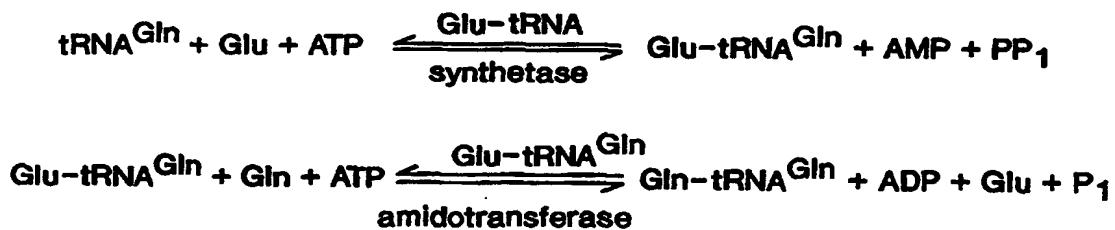


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(71) Applicant (for all designated States except US): YALE UNIVERSITY [US/US]; 451 College Street, New Haven, CT 06520 (US).			
(72) Inventor; and (75) Inventor/Applicant (for US only): SÖLL, Dieter [US/US]; 145 Dessa Drive, Hamden, CT 06517 (US).			
(74) Agents: ADLER, Reid, G. et al.; Morgan, Lewis & Bockius LLP, 1800 M Street, N.W., Washington, DC 20036 (US).			

(54) Title: GLUTRNA<sup>GLN</sup> AMIDOTRANSFERASE – A NOVEL ESSENTIAL TRANSLATIONAL COMPONENT

## (57) Abstract

The present method provides the amino acid sequence and encoding nucleic acid sequence of GlutRNA<sup>GLN</sup> amidotransferase (AdT), a protein that is essential for protein translation. The AdT proteins and encoding nucleic acid molecules herein described can be used as targets for identifying agents that block translations. Such agents can be used as an antimicrobial, antifungal or herbicide agent.

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**GlutRNA<sup>Gln</sup> Amidotransferase - A Novel Essential Translational Component****CROSS REFERENCE TO RELATED APPLICATIONS**

This application is related to U.S. Provisional Application Serial No. 60/037,275; filed February 3, 1997, which is herein incorporated by reference in its entirety.

**FIELD OF THE INVENTION**

The present invention is in the field of inhibitors of protein translation, particularly translation of proteins within microorganisms and organelles. This invention relates to newly identified polynucleotides and polypeptides, and their 5 production and uses, as well as their variants, agonists and antagonists, and their uses. In particular, in these and in other regards, the invention relates to novel polynucleotides and polypeptides of the Glu-tRNA<sup>Gln</sup> Amidotransferase family, hereinafter referred to as "Glu-tRNA<sup>Gln</sup> AdT" or "AdT". The present invention further provides methods and compositions for use in identifying and using protein 10 translation inhibitors as antibacterial, antifungal or herbicidal agents.

**BACKGROUND OF THE INVENTION**

Prior to their incorporation into protein, amino acids are chemically linked to small RNA molecules called transfer RNA (tRNA). For each of the 20 different amino acids, a specific enzyme catalyzes its linkage to the 3' end of its specific tRNA 15 molecule. While the general mechanism of protein biosynthesis (the translation

process) is conserved throughout the living kingdom there exist two different pathways for the formation of Gln<sup>tRNA<sub>Gln</sub></sup>. While the two pathways for Gln<sup>tRNA<sub>Gln</sub></sup> formation are evolutionarily conserved, the reason for existence of the different pathways is as yet not known. In gram-negative eubacteria and in the cytoplasm of 5 eukaryotic cells the enzyme glutaminyl-tRNA synthetase (GlnRS) acylates glutamine directly to the cognate tRNA to provide Gln<sup>tRNA<sub>Gln</sub></sup>. Interestingly, GlnRS is not detectable in several biological systems. In certain organisms and organelles including the archae, gram-positive eubacteria, mitochondria and chloroplasts a different pathway of Gln<sup>tRNA<sub>Gln</sub></sup> formation, a transamidation pathway is operative 10 (Curnow *et al.* (1996) *Nature* 382: 589-590; Curnow *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94(22):11819-11826; Schön *et al.* (1988) *Biochimie* 70(3):391-394; Wilcox & Nirenberg (1968) *Proc. Natl. Acad. Sci. USA* 61(1):229-236; Schön *et al.* (1988) *Nature* 331:187-190. This pathway (depicted in Figure 1) is initiated by misacylation 15 of tRNA<sup>Gln</sup> by glutamyl-tRNA synthetase (GluRS) forming GlutRNA<sup>Gln</sup>. The incorrectly charged tRNA is then converted to Gln<sup>tRNA<sub>Gln</sub></sup> by GlutRNA<sup>Gln</sup> amidotransferase (AdT). AdT catalyzes the amidation of glutamate to glutamine only when the glutamate is covalently attached to tRNA<sup>Gln</sup>. It has been shown that the partially purified GlutRNA<sup>Gln</sup> amidotransferase activity from *Bacillus megaterium* in the presence of ATP, Mg<sup>++</sup>, and an amide-nitrogen donor (glutamine) will carry out 20 the amidation of GlutRNA<sup>Gln</sup> to Gln<sup>tRNA<sub>Gln</sub></sup> (Wilcox & Nirenberg, 1968).

Subsequent work demonstrated, in vitro, that the amidation proceeds through the activated intermediate (phospho-GlutRNA<sup>Gln</sup>) (Wilcox (1969) *Cold Spring Harb. Symp. Quant. Biol.* 34:521-528; Wilcox (1969) *Eur J. Biochem* 11(3):405-412). Since the initial aminoacylation product, GlutRNA<sup>Gln</sup>, would be toxic to the cell due to the 5 fact that it would result in faulty protein translation, it must be converted to the correctly charged tRNA.

It appears that this pathway is the primary source of GlntRNA<sup>Gln</sup> within these cells and may act as a regulatory mechanism for glutamine metabolism. Evolutionarily, it has been suggested that glutamine was the last amino acid formed. 10 Therefore it may be postulated that cells which employ the transamidation pathway utilized the gene encoding GluRS to generate the AdT. Likewise, in the cells in which the direct glutaminylation pathway operates, the enzyme GlnRS may have evolved from a GluRS gene duplication (Rogers & Söll (1995) *J. Mol. Evol.* 40 (5) p476-81). This is reasonable since both enzymes are required to specifically 15 recognize and bind tRNAGln and free glutamine. However, database searches and, in particular, a detailed analysis of the Mycoplasma genome (Fraser et al. (1995) *Science* 270(5235):397-403), the only gram-positive organism sequenced and published to date, have shown no significant homologies to GluRS and GlnRS in the currently 20 available sequence information. Thus, the amidotransferase may not have significant homology to the aminoacyl-tRNA synthetases. Despite the unquestioned

evolutionary and biochemical significance in understanding this system, there have been very few investigations of this enzyme to date (Wilcox & Nirenberg, 1968; Wilcox, 1969; Strauch et al. (1988) *J. Bacteriol.* 170:916-920; and Jahn (1990) *J. Biol. Chem.* 265(14):8059-64).

## 5 SUMMARY OF THE INVENTION

The present invention is based, in part, on the isolation and characterization of a heterotrimeric protein designated AdT that is involved in generating GlntRNA<sup>Gln</sup> from GlutRNA<sup>Gln</sup>. This invention further provides polypeptides that have been identified as novel AdT polypeptides by homology between the amino acid sequence 10 of GlutRNA<sup>Gln</sup> AdT and a known amino acid sequence.

This invention further provides polynucleotides that encode AdT polypeptides. In particular, this invention provides the polynucleotide sequence encoding GlutRNA<sup>Gln</sup> AdT comprising the sequence set out in Figure 3 (SEQ ID NO:1), or a variant thereof, such as naturally occurring allelic variants of AdT and 15 polypeptides encoded thereby. Thus, this invention provides polynucleotides that hybridize to AdT polynucleotide sequences, particularly under stringent conditions.

This invention provides GlutRNA<sup>Gln</sup> AdT protein from *B. subtilis* comprising the amino acid sequences encoded by the nucleotide sequence of Figure 3 (SEQ ID NOS:1, 3, 5 and 7), as well as biologically, diagnostically, prophylactically, clinically

or therapeutically useful variants thereof, and compositions comprising the same.

Particularly preferred variants include AdT polypeptides encoded by naturally occurring alleles of the AdT gene. Methods for producing the aforementioned AdT polypeptides are also provided by this invention.

5 The invention also provides isolated nucleic acid molecules encoding AdT, particularly *B. subtilis* AdT, including mRNAs, cDNAs, and genomic DNAs, including biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

In accordance with yet another aspect of the invention, there are provided  
10 inhibitors to such AdT polypeptides, useful as antibacterial agents, antifungal agents and herbicides. Thus, the present invention provides compositions and methods for use in identifying agonists and antagonists of the AdT protein.

This invention provides compositions and methods for (i) assessing AdT expression, (ii) treating disease, for example, diseases associated with excessive or  
15 deficient amounts of available AdT, (iii) assaying genetic variation, and (iv) and administering an AdT polypeptide or polynucleotide to a cell or to a multicellular organism to raise an immunological response. In certain preferred embodiments of this aspect of the invention there are provided antibodies against AdT polypeptides.

This invention also provides compositions and methods for protecting plants,  
20 especially crop plants. For example, this invention provides antagonists of AdT

which are useful as herbicides, as well as the herbicidal compositions which include such inhibitors of AdT. This invention also provides non-inhibited mutants of AdT and functional derivatives thereof which are resistant to inhibition from certain herbicides, especially herbicides containing inhibitors of AdT. The polynucleotides 5 coding for the non-inhibited AdT can be placed in plants by various transformation methods so as to render the plants tolerant or resistant to certain herbicides containing inhibitors of AdT. Therefore, methods of treating weeds utilizing the application of AdT inhibitors to transgenic plants containing the non-inhibited mutants of AdT are also encompassed by this invention.

10 Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows the transamidation pathway for the formation of GlntRNA<sup>Gln</sup>.

15 Figure 2 shows the gene arrangement of the AdT gene.

Figure 3 shows the nucleic acid sequence of the AdT protein from *B. subtilis*.

#### **DESCRIPTION OF THE INVENTION**

##### **I. General Description**

The present invention is based, in part, on the identification and characterization of a heterotrimeric protein that is responsible for generating GlntRNA within a cell, herein after the AdT protein. The present invention specifically provides the amino acid sequences of each of the three subunits of an AdT protein isolated from *B. subtilis*, as well as nucleotide sequences that encode the AdT protein. The AdT protein and nucleic acid molecules can serve as targets in methods for identifying agents for use in inhibiting protein synthesis, particularly antimicrobial, antifungal and herbicide agents.

## II. Specific Embodiments

### 10 A. AdT Protein

Prior to the present invention the art had taught that there was an enzyme involved in converting GlutRNA<sup>Gln</sup> to GlntRNA<sup>Gln</sup>. However the isolation and characterization of the protein responsible for generating GlntRNA remained unknown. The present invention provides, in part, the amino acid sequences of the three subunits of the *B. subtilis* AdT protein. Quite unexpectedly, this AdT protein was found to be a heterotrimeric protein.

In one embodiment, the present invention provides the ability to produce a previously unknown protein using the cloned nucleic acid molecules herein described or by synthesizing a protein having the amino acid sequence herein disclosed.

As used herein, the AdT protein refers to a protein that has the amino acid sequence *B. subtilis* AdT encoded by the polynucleotide of Fig. 1, allelic variants thereof and conservative substitutions thereof that have AdT activity. The AdT protein is comprised of 3 subunits: the A (SEQ ID NO:4), B (SEQ ID NO:6) and C (SEQ ID NO:8) subunits, referred to herein collectively as aAdT, bAdT and cAdT subunits, respectively. For the sake of convenience, the collective subunits will be referred to as the AdT protein or the AdT protein of the present invention. A skilled artisan can readily recognize within the context whether a single subunit or the collective protein is being referred to.

10 The polypeptides of the invention include the polypeptides encoded by SEQ ID NO:1 (Figure 3) as well as polypeptides and fragments, particularly those which have the biological activity of AdT and also those which have at least 70% sequence identity to the polypeptides encoded by SEQ ID NO:1 or the relevant portion, preferably at least 80% identity to the polypeptides encoded by SEQ ID NO:1, and 15 more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptides encoded by SEQ ID NO:1 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptides encoded by SEQ ID NO:1 and also include portions of such polypeptides.

20 The AdT proteins of the present invention include the specifically identified and characterized variant herein described as well as allelic variants, conservative

substitution variants and homologues that can be isolated/generated and characterized without undue experimentation following the methods outlined below. For the sake of convenience, all AdT proteins will be collectively referred to as the AdT proteins or the AdT proteins of the present invention.

5           The term "AdT proteins" includes all naturally occurring allelic variants of the *B. subtilis* AdT protein that possess normal AdT activity. In general, allelic variants of the AdT protein will have a slightly different amino acid sequence than that specifically encoded by SEQ ID NO:1 but will be able to convert GlutRNA to GlntRNA. Allelic variants, though possessing a slightly different amino acid sequence than those recited above, will possess the ability to generate GlntRNA.

10           Typically, allelic variants of the AdT protein will contain conservative amino acid substitutions from the AdT sequences herein described or will contain a substitution of an amino acid from a corresponding position in an AdT homologue (an AdT protein isolated from an organism other than *B. subtilis*).

15           The AdT proteins of the present invention are preferably in isolated form. As used herein, a protein is said to be isolated when physical, mechanical or chemical methods are employed to remove the AdT protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated AdT protein. One purification scheme is

-10-

outlined in Example 1. The nature and degree of isolation will depend on the intended use.

The cloning of an AdT encoding nucleic acid molecule makes it possible to generate defined fragments of the AdT proteins of the present invention. As discussed 5 below, fragments of the AdT proteins of the present invention are particularly useful in generating subunit specific antibodies, in identifying agents that bind to a AdT protein and in isolating homologues of the *B. subtilis* AdT protein.

Fragments of the AdT proteins can be generated using standard peptide synthesis technology and the amino acid sequences disclosed herein. Alternatively, 10 recombinant methods can be used to generate nucleic acid molecules that encode a fragment of the AdT protein.

Fragments of the AdT protein subunits that contain particularly interesting structures can be identified using art-known methods such as immunogenicity, Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or 15 Jameson-Wolf analysis. Fragments containing such residues are particularly useful in generating subunit specific anti-AdT antibodies.

As described below, members of the AdT family of proteins can be used for, but are not limited to: 1) a target to identify agents that block or stimulate AdT activity, 2) a target or bait to identify and isolate binding partners that bind an AdT

protein, 3)identifying agents that block or stimulate the activity of an AdT protein and 4) an assay target to identify AdT mediated activity or disease.

#### **B. Anti-AdT Antibodies**

The present invention further provides antibodies that selectively bind one or 5 more of the AdT proteins of the present invention, or to a specific subunit of an AdT protein of the present invention. The most preferred antibodies will bind to either an entire heterotrimeric AdT protein but not to an isolated subunit or will bind to an isolated subunit but not to the assembled trimeric protein. Anti- AdT antibodies that are particularly contemplated include monoclonal and polyclonal antibodies as well as 10 fragments containing the antigen binding domain and/or one or more complement determining regions of these antibodies.

Antibodies are generally prepared by immunizing a suitable mammalian host using an AdT protein, or fragment, in isolated or immunoconjugated form (Harlow, Antibodies, Cold Spring Harbor Press, NY (1989)). Regions of the AdT protein that 15 show immunogenic structure can readily be identified using art-known methods. Other important regions and domains can readily be identified using protein analytical and comparative methods known in the art.

Fragments containing these residues are particularly suited in generating specific classes of anti-AdT antibodies.

Methods for preparing a protein for use as an immunogen and for preparing immunogenic conjugates of a protein with a carrier such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be used; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be effective.

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Administration of an AdT immunogen is conducted generally by injection over a suitable time period and with use of a suitable adjuvant, as is generally understood in the art. During the immunization schedule, titers of antibodies can be

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taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, monoclonal antibody preparations are preferred. Immortalized cell lines which secrete a desired monoclonal antibody may be prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the AdT protein or peptide fragment. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either in vitro or by production

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in ascites fluid.

-13-

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive 5 fragments, such as the Fab, Fab', of F(ab')2 fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of the 10 transporter can also be produced in the context of chimeric or CDR grafted antibodies of multiple species origin.

As described below, anti-AdT antibodies are useful as modulators of AdT activity, are useful in immunoassays for detecting AdT expression/activity and for purifying homologues of the *B. subtilis* AdT protein.

15      **C.      AdT Encoding Nucleic Acid Molecules**

As described above, the present invention is based, in part, on isolating nucleic acid molecules from *B. subtilis* that encode the three subunits of the AdT protein. Accordingly, the present invention further provides nucleic acid molecules that encode the AdT protein, as herein defined, preferably in isolated form. For

-14-

convenience, all AdT encoding nucleic acid molecules will be referred to as AdT encoding nucleic acid molecules, the AdT genes, or AdT. The nucleotide sequence of the *B. subtilis* nucleic acid molecule that encodes each of the subunits of AdT is provided in SEQ ID NO:1. The start and stop codons for each of subunits A (SEQ 5 ID NO:4), B (SEQ ID NO:6) and C (SEQ ID NO:8) are designated in the nucleotide sequence for AdT provided in Figure 3.

Further preferred embodiments of the invention are polynucleotides that are at least 70% sequence identical over their entire length to a polynucleotide encoding AdT polypeptides having an amino acid sequence encoded by SEQ ID NO:1, and 10 polynucleotides which are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical over their entire length to a polynucleotide encoding AdT polypeptide and polynucleotides complementary thereto. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among 15 these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

The invention further relates to variants of the herein above described polynucleotides which encode for variants of the polypeptides having the deduced amino acid sequences of SEQ ID NO:1.

Variants that are fragments of the polypeptides of the invention may be 5 employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides. Variants that are fragments of the polynucleotides of the invention may be used to synthesize full-length polynucleotides of the invention.

Variants that are fragments of the polynucleotides of the invention may be used to 10 synthesize full-length polynucleotides of the invention. Such methods are widely available, such as those disclosed in WO 97/26340 and WO 97/38716.

A fragment is a variant polypeptide having an amino acid sequence that 15 entirely is the same as part but not all of the amino acid sequence of the aforementioned polypeptides. AdT polypeptides fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region, a single larger polypeptide.

Further particularly preferred embodiments are polynucleotides encoding AdT variants, which have the amino acid sequence of the AdT polypeptides encoded by 20 SEQ ID NO:1 in which several, a few, 10 to 15, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially

preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of AdT.

As used herein, a "nucleic acid molecule" is defined as an RNA or DNA molecule that encodes a peptide as defined above, or is complementary to a nucleic acid sequence encoding such peptides. Particularly preferred nucleic acid molecules will have a nucleotide sequence identical to or complementary to the *B. subtilis* DNA sequences herein disclosed. Specifically contemplated are genomic DNA, polycistronic mRNA and antisense molecules, as well as nucleic acids based on an alternative backbone or including alternative bases, whether derived from natural sources or synthesized. Such nucleic acid molecules, however, are defined further as being novel and unobvious over any prior art nucleic acid molecules encoding non-AdT proteins isolated from organisms other than *B. subtilis*.

As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid molecules that encode polypeptides other than AdT. A skilled artisan can readily employ nucleic acid isolation procedures to obtain an isolated AdT encoding nucleic acid molecule.

The present invention further provides fragments of the AdT encoding nucleic acid molecules of the present invention. As used herein, a fragment of an AdT encoding nucleic acid molecule refers to a small portion of the entire protein encoding

sequence. The size of the fragment will be determined by its intended use. For example, if the fragment is chosen so as to encode an active portion of the AdT protein, such an active domain or effector binding site, then the fragment will need to be large enough to encode the functional region(s) of the AdT protein. If the fragment 5 is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming. Fragments of the *B. subtilis* AdT nucleic acid molecule that are particularly useful as selective hybridization probes or PCR can be readily determined using art-known methods.

10                   Fragments of the AdT encoding nucleic acid molecules of the present invention (i.e., synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding AdT proteins, can easily be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, et al., *J Am Chem Soc* (1981) 103:3185-3191 or 15 using automated synthesis methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the AdT gene, followed by ligation of oligonucleotides to build the complete modified AdT gene.

20                   The AdT encoding nucleic acid molecules of the present invention may further be modified so as to contain a detectable label for diagnostic and probe purposes. As

-18-

described above, such probes can be used to identify nucleic acid molecules encoding other allelic variants or homologues of the AdT proteins and as described below, such probes can be used to diagnose the presence of a AdT protein as a means for diagnosing a pathological condition caused by AdT mediated translation. A variety of 5 such labels are known in the art and can readily be employed with the AdT encoding molecules herein described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides, biotin, and the like. A skilled artisan can employ any of the art-known labels to obtain a labeled AdT encoding nucleic acid molecule.

#### **D. Isolation of Other AdT Encoding Nucleic Acid Molecules**

10 The identification of the AdT protein from *B. subtilis* and the corresponding nucleic acid molecules, has made possible the identification of and isolation of AdT proteins from organisms other than *B. subtilis*, hereinafter referred to collectively as AdT homologues. The preferred source of the AdT homologues are pathogenic microorganisms such as bacteria and fungi, as well as plants in which it is desirable to 15 control growth. The most preferred sources are gram positive bacteria, pathogenic fungi and plant organelles such as chloroplasts.

Essentially, a skilled artisan can readily use the amino acid sequence of the *B. subtilis* AdT protein to generate antibody probes to screen expression libraries prepared from cells. Typically, polyclonal antiserum from mammals such as rabbits

immunized with the purified protein (as described below) or monoclonal antibodies can be used to probe an expression library, prepared from a target organism, to obtain the appropriate coding sequence for AdT protein homologue. The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own 5 control sequences, or expressed by constructing an expression cassette using control sequences appropriate to the particular host used for expression of the enzyme.

Alternatively, a portion of the AdT encoding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the AdT family of proteins from organisms other than *B. subtilis*. Oligomers containing 10 approximately 18-20 nucleotides (encoding about a 6-7 amino acid stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient stringency to eliminate an undue level of false positives. This method can be used to identify and isolate altered and variants of the AdT encoding sequences.

15        Additionally, pairs of oligonucleotide primers can be prepared for use in a polymerase chain reaction (PCR) to selectively amplify/clone an AdT-encoding nucleic acid molecule, or fragment thereof. A PCR denature/anneal/extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other AdT encoding nucleic acid molecules. Regions of the *B. subtilis* AdT 20 gene that are particularly well suited for use as a probe or as primers can be readily

identified. In general, the preferred primers will flank one or more of the subunit encoding regions of the *B. subtilis* AdT gene.

Homologues of the herein disclosed AdT proteins will share homology. In general, nucleic acid molecules that encode AdT homologues will hybridize to the 5 *B. subtilis* sequences under high stringency. Such sequences will typically contain at least 70% homology, preferably at least 80%, most preferably at least 90% homology to the *B. subtilis* sequences.

#### **E. Recombinant DNA Molecules Containing an AdT Encoding Nucleic Acid Molecule**

10 The present invention further provides recombinant DNA molecules that contain one or more of the AdT encoding sequences herein described, or a fragment of the herein-described nucleic acid molecules. As used herein, an recombinant DNA molecule is a DNA molecule that has been subjected to molecular manipulation in vitro. Methods for generating recombinant DNA molecules are well known in the art, 15 for example, see Sambrook et al., *Molecular Cloning* (1989). In the preferred recombinant DNA molecules, an AdT encoding DNA sequence that encodes an AdT protein, or AdT subunit is operably linked to one or more expression control sequences and/or vector sequences. The recombinant DNA molecule can encode

either a single subunit of the AdT protein, or can encode an operon that contains all three of the AdT subunits.

The choice of vector and/or expression control sequences to which one of the AdT encoding sequences of the present invention is operably linked depends directly, 5 as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication or insertion into the host chromosome, and preferably also expression, of an AdT encoding sequence included in the recombinant DNA molecule.

10 Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, enhancers, transcription terminators and other regulatory elements. Preferably, an inducible promoter that is readily controlled, such as being responsive to a nutrient in 15 the host cell's medium, is used.

In one embodiment, the vector containing an AdT encoding nucleic acid molecule will include a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule intrachromosomally in a prokaryotic host cell, such as a bacterial host cell, 20 transformed therewith. Such replicons are well known in the art. In addition, vectors

that include a prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Vectors that include a prokaryotic replicon can further include a prokaryotic or 5 viral promoter capable of directing the expression (transcription and translation) of the AdT encoding sequence in a bacterial host cell, such as *E. coli*. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites 10 for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories (Richmond, CA), pPL and pKK223 available from Pharmacia, Piscataway, NJ.

Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can also be used to variant recombinant DNA 15 molecules that contain an AdT encoding sequence. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment. Typical of such vectors are PSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), pTDT1

(ATCC, #31255), the vector pCDM8 described herein, and the like eukaryotic expression vectors.

Eukaryotic cell expression vectors used to construct the recombinant DNA molecules of the present invention may further include a selectable marker that is 5 effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (neo) gene. Southern et al., *J Mol Anal Genet* (1982) 1:327-341. Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors are introduced by cotransfection of the host 10 cell, and selected by culturing in the presence of the appropriate drug for the selectable marker.

**F. Host Cells Containing an Exogenously Supplied AdT Encoding Nucleic Acid Molecule**

The present invention further provides host cells transformed with a nucleic 15 acid molecule that encodes an AdT protein of the present invention, either the entire heterotrimeric protein or one or more subunits. The host cell can be either prokaryotic or eukaryotic. Eukaryotic cells useful for expression of an AdT protein are not limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of an AdT

gene. Preferred eukaryotic host cells include, but are not limited to, yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line, the most preferred being cells that do not naturally express an AdT protein.

5 Any prokaryotic host can be used to express an AdT-encoding recombinant DNA molecule. The preferred prokaryotic host is *E. coli*.

Transformation of appropriate cell hosts with an recombinant DNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. With regard to transformation 10 of prokaryotic host cells, electroporation and salt treatment methods are typically employed, see, for example, Cohen et al., *Proc Acad Sci USA* (1972) 69:2110; and Maniatis et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor 15 Laboratory, Cold Spring Harbor, NY (1982). With regard to transformation of vertebrate cells with vectors containing recombinant DNAs, electroporation, cationic lipid or salt treatment methods are typically employed, see, for example, Graham et al., *Virol* (1973) 52:456; Wigler et al., *Proc Natl Acad Sci USA* (1979) 76:1373-76.

Successfully transformed cells, i.e., cells that contain an recombinant DNA molecule of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an recombinant DNA of the present 20 invention can be cloned to produce single colonies. Cells from those colonies can be

harvested, lysed and their DNA content examined for the presence of the recombinant DNA using a method such as that described by Southern, *J Mol Biol* (1975) 98:503, or Berent et al., *Biotech* (1985) 3:208 or the proteins produced from the cell assayed via an immunological method.

5       **G.      Production of an AdT Protein Using an recombinant DNA Molecule**

The present invention further provides methods for producing an AdT protein that uses one of the AdT encoding nucleic acid molecules herein described. In general terms, the production of a recombinant AdT protein typically involves the following steps.

10       First, a nucleic acid molecule is obtained that encodes an AdT protein, such as the nucleic acid molecule depicted in Figure 3 (SEQ ID NO:1) or an AdT subunit. The AdT encoding nucleic acid molecule is then preferably placed in an operable linkage with suitable control sequences, as described above, to generate an expression unit containing the AdT encoding sequence. The expression unit is used to transform 15 a suitable host and the transformed host is cultured under conditions that allow the production of the AdT protein. Optionally the AdT protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

Each of the foregoing steps can be done in a variety of ways. For example, the desired coding sequences may be obtained from genomic fragments and used directly in an appropriate host. The construction of expression vectors that are operable in a variety of hosts is accomplished using an appropriate combination of replicons and 5 control sequences. The control sequences, expression vectors, and transformation methods are dependent on the type of host cell used to express the gene and were discussed in detail earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system 10 known in the art for use with AdT encoding sequences to produce an AdT protein.

#### **H. Identification of Agents that Bind to an AdT Protein**

Another embodiment of the present invention provides methods for identifying agents that are agonists or antagonists of the AdT proteins herein described. Specifically, agonists and antagonists of an AdT protein can be identified by the 15 ability of the agent to bind to an AdT protein and/or the ability to inhibit AdT activity. Activity assays for AdT activity and binding assays using an AdT protein are suitable for use in high through-put screening methods.

In detail, in one embodiment, an AdT protein is mixed with an agent. After mixing under conditions that allow association of AdT protein with the agent, the

mixture is analyzed to determine if the agent bound the AdT protein. Agonists and antagonists are identified as being able to bind to an AdT protein. Alternatively or consecutively, as described below, AdT activity can be directly assessed as a means for identifying agonists and antagonists of AdT activity.

5        The AdT protein used in the above assay can be: an isolated and fully characterized protein, a single subunit of an AdT protein, a partially purified protein, a cell that has been altered to express an AdT protein or a fraction of a cell that has been altered to express an AdT protein. Further, the AdT protein can be the entire AdT protein, a specific fragment of the AdT protein or a single subunit of the AdT protein.

10      It will be apparent to one of ordinary skill in the art that so long as the AdT protein can be assayed for agent binding, e.g., by a shift in molecular weight or activity, as described in the Examples, the present assay can be used. The AdT protein is particularly well suited for high through-put screening methods.

15      The source of the AdT protein will be based on the intended use of the modulating agent. For example, microbial AdT protein is used to identify AdT inhibitors that have bactericidal activity whereas chloroplast derived AdT protein is used to identify AdT inhibitors that have herbicide activity.

20      The method used to identify whether an agent binds to an AdT protein will be based primarily on the nature of the AdT protein used. For example, a gel retardation assay can be used to determine whether an agent binds to a soluble fragment of an

-28-

AdT protein. Alternatively, immunodetection and biochip technologies can be adopted for use with an AdT protein. A skilled artisan can readily employ numerous art-known techniques for determining whether a particular agent binds to an AdT protein.

5 Agents can be further tested for the ability to modulate the activity of an AdT protein using a cell-free assay system or a cellular assay system. Example 1 provides one such methods that can be used to assay for AdT activity.

As used herein, an agent is said to antagonize AdT activity when the agent reduces AdT activity. The preferred antagonist will selectively antagonize AdT, not 10 affecting any other cellular proteins, particularly other proteins involved in translation. Further, the preferred antagonist will reduce AdT activity by more than 50%, more preferably by more than 90%, most preferably eliminating all AdT activity.

As used herein, an agent is said to agonize AdT activity when the agent increases AdT activity. The preferred agonist will increase AdT activity by more than 15 50%, more preferably by more than 90%, most preferably more than doubling the level of AdT activity.

The preferred antagonists and agonists will be selective for a specific species, genus, family, order or kingdom of organisms. Agents can be screened using one AdT protein, or a combination of AdT proteins, to aid in identifying agents for target 20 specificity. For example, several different microbial AdT proteins can be used to

identify general antimicrobial agents whereas chloroplast derived AdT proteins can be used to identify herbicide agents.

Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences of the AdT protein. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism or plant extract.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis that takes into account the sequence of the target site and/or its conformation in connection with the agent's action. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up the AdT protein. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to a fragment of an AdT protein.

The agents of the present invention can be, as examples, peptides, small molecules, and vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention. One class of agents of the present invention are peptide agents whose amino acid sequences are chosen based on the amino acid sequence of the AdT

-30-

protein. Small peptide agents can serve as competitive inhibitors of AdT protein assembly.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the 5 DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents of the present invention are antibodies immunoreactive 10 with critical positions of the AdT protein. As described above, antibodies are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the AdT protein intended to be targeted by the antibodies. Critical regions include the domains identified in Figure 2. Such agents can be used in competitive binding studies to identify second generation inhibitory 15 agents.

#### **K. Uses of Agents that Bind to an AdT Protein**

As provided in the Background section, the AdT proteins are involved in protein translation, particularly protein translation in gram positive microorganisms, fungi and cellular organelles, particularly chloroplasts. Agents that bind an AdT

-31-

protein and act as an agonist or antagonist can be used to modulate translation in these organism and serves as a basis for an antibacterial, antifungal or herbicide agents. In detail, protein translation that requires AdT can be modulated by administering to an organism an agent that binds to an AdT protein and acts as an agonist or antagonist of

5 AdT activity.

As used herein, an organism can be any organism, so long as it is desirable to modulate protein translation in the organism, for example to control the growth of an infectious agent in a mammalian subject or to act as an herbicide agent. The invention is particularly useful in the treatment of human subjects for controlling microbial 10 growth.

As used herein, protein translation that requires AdT refers to protein translation that would not occur without the presence of an active AdT protein. As used herein, an agent is said to modulate AdT mediated protein translation when the agent reduces the degree of protein translation.

15 The use of the AdT modulating agents will be based primarily on the target AdT protein used to identify the agent as well as the activity/selectivity of the agent. For example, an AdT inhibitory agent, that is used as an antimicrobial agent, is preferably isolated using one or more microbial AdT proteins. Herbicide agent will be preferably identified using chloroplast AdT protein as a target.

#### **L. Administration of Agonists and Antagonists of an AdT Protein**

The administration of agonists and antagonists of the AdT protein will be dependent on their intended purpose. For example, to control microbial growth in a mammalian subject, an AdT inhibitory agent can be administered via parenteral, 5 subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. For example, to treat microbial infection, an agent that 10 modulates AdT activity is administered systemically or locally to the individual being treated. As described below, there are many methods that can readily be adapted to administer such agents.

The present invention further provides compositions containing an antagonist or agonist of an AdT protein that is identified by the methods herein described. The 15 determination of optimal ranges of effective amounts of each component is within the skill of the art and is based on the intended use.

In addition to the AdT modulating agent, the compositions of the present invention may contain other ingredients, such as suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active 20 compounds into preparations which can be used pharmaceutically for delivery to the

site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble variant, for example, water-soluble salts. In addition, suspensions of the active compounds and as appropriate, oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles 5 include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dintran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for 10 delivery into the cell.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

15 Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release variants thereof.

#### **M. Combination Therapy**

The agents of the present invention that modulate AdT activity can be provided alone, or in combination with another agents that modulate protein synthesis microbial, fungal or plant growth. For example, an agent of the present invention that reduces microbial AdT activity can be administered in combination with other 5 antimicrobial agents. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act at the same time.

#### **N. Methods for Identifying the Presence of an AdT protein or gene**

The present invention further provides methods for identifying cells or 10 organisms expressing an AdT protein or an AdT gene. Such methods can be used to diagnose the presence of an organism that expresses an AdT protein. The methods of the present invention are particularly useful in the determining the presence of pathogenic microorganisms. Specifically, the presence of an AdT protein can be identified by determining whether an AdT protein, or nucleic acid encoding an AdT 15 protein, is expressed. The expression of an AdT protein can be used as a means for diagnosing the presence of an organism that relies on AdT mediated translation.

A variety of immunological and molecular genetic techniques can be used to determine if an AdT protein is expressed/produced in a particular cell or sample. In general, an extract containing nucleic acid molecules or an extract containing proteins

is prepared. The extract is then assayed to determine whether an AdT protein, or an AdT encoding nucleic acid molecule, is produced in the cell.

For example, to perform a diagnostic test based on nucleic acid molecules, a suitable nucleic acid sample is obtained and prepared using conventional techniques. 5 DNA can be prepared, for example, simply by boiling a sample in SDS. The extracted nucleic acid can then be subjected to amplification, for example by using the polymerase chain reaction (PCR) according to standard procedures, to selectively amplify an AdT encoding nucleic acid molecule or fragment thereof. The size or presence of a specific amplified fragment (typically following restriction 10 endonuclease digestion) is then determined using gel electrophoresis or the nucleotide sequence of the fragment is determined (for example, see Weber and May *Am J Hum Genet* (1989) 44:388-339; Davies, J. et al. *Nature* (1994) 371:130-136)). The resulting size of the fragment or sequence is then compared to the known AdT 15 proteins encoding sequences, for example via hybridization probe. Using this method, the presence of an AdT protein can be identified.

To perform a diagnostic test based on proteins, a suitable protein sample is obtained and prepared using conventional techniques. Protein samples can be prepared, for example, simply by mixing a sample with SDS followed by salt 20 precipitation of a protein fraction. The extracted protein can then be analyzed to determine the presence of an AdT protein using known methods. For example, the

presence of specific sized or charged variants of a protein can be identified using mobility in an electric field. Alternatively, antibodies can be used for detection purposes. A skilled artisan can readily adapt known protein analytical methods to determine if a sample contains an AdT protein.

5        Alternatively, AdT expression can also be used in methods to identify agents that decrease the level of expression of the AdT gene. For example, cells or tissues expressing an AdT protein can be contacted with a test agent to determine the effects of the agent on AdT expression. Agents that activate AdT expression can be used as an agonist of AdT activity whereas agents that decrease AdT expression can be used  
10      as an antagonist of AdT activity.

#### **O. Preparation and Use of Herbicides**

As discussed herein, the transamidation pathway is operative in chloroplasts. The ability to identify AdT inhibitors which specifically inhibit plastid isoforms of AdT can be useful in designing herbicides that are not toxic or harmful to humans and animals. Thus, the ability to develop herbicides that inhibit only chloroplast isoforms  
15      of enzymes such as Adt but do not inhibit cytosolic (i.e., the fluid portion of the cytoplasm exclusive of organelles) AdT or human AdT, would provide a new form of highly effective herbicide that is also less toxic to humans. However, Adt inhibitors which are not limited to the chloroplasts may also find utility in use as an herbicide.

An identified compound which inhibits function of the wild-type AdT enzyme is utilized as an active ingredient in an herbicide. The active ingredient is normally applied in the form of compositions together with one or more agriculturally acceptable carriers, and can be applied to the crop area or plant to be treated, 5 simultaneously or in succession, with further compounds. These additional compounds can include fertilizers, other herbicides, fungicides, bactericides, nematicides, or mixtures of several of these preparations, together with further carriers, surfactants or application-promoting adjuvants. The herbicide may be applied as a seed coating, a ground spray, incorporated into the soil, or applied 10 directly to the plant. Preferably, the active ingredient of the present invention or an agrochemical composition which contains at least one of the active ingredients of the present invention are applied as a leaf preparation. Methods of herbicide preparation and application are well known to one skilled in the art.

Resistant mutants to the AdT-inhibiting compound can be identified by 15 mutagenizing cells or organisms and growing the mutagenized populations in the presence of a concentration of the inhibitor sufficient to inhibit growth of the wild-type cells or organisms, and selecting cells or organisms from the populations that are able to grow more rapidly than wild-type cells or organisms. Mutagenesis can be accomplished by any one of the means well known to one skilled in the art, including: 20 chemical mutagenesis (e.g., ethyl methanesulfonate); ultraviolet radiation; X-ray

exposure; and gamma radiation (see, e.g., Watson *et al.*, *Recombinant DNA, Second Edition* (1992) Chapter 11:191-211; Freifelder, *Molecular Biology* (1987) Chapter 11:293-313). The mutant individuals which have the ability to tolerate or resist the normally toxic levels of the inhibitor are genetically purified, the gene encoding the 5 mutant AdT is isolated, and the DNA sequence of the mutant gene is determined and translated into a predicted amino acid sequence. The amino acids which differ between the wild-type AdT enzyme and the mutant AdT enzyme are assumed to be responsible for the inhibitor-resistant phenotype of the newly-identified mutant.

The coding DNA sequence for the mutant AdT can be introduced into the 10 plant cell in a number of different ways that are well known to those of skill in the art. Examples of such methods include micro injection, electroporation, Agrobacterium-mediated transformation, direct gene transfer, and micro projectile bombardment. Techniques for producing herbicide resistance in plants by incorporating DNA 15 encoding and expressing enzymes resistant to herbicides are well known (see, e.g., U.S. Patent No. 5,145,777; U.S. Patent No. 5,290,926), including techniques for adding a chloroplast transit sequence upstream from an herbicide gene so that the protein product is transported into the chloroplasts (Comai *et al.*, *Nature* (1985) 313:741-744; U.S. Patent No. 4,940,835; U.S. Patent No. 5,188,642). In the same manner, the gene coding for a mutant AdT may be substituted for one of the other 20 herbicide resistance genes of the references. Since AdT performs its function in the

chloroplast, it may be particularly relevant to use a plastid transit sequence to ensure expression in the chloroplast or other plastid as is known in the art.

Following introduction of the mutant AdT gene into plant cells and the regeneration of transformed plants from such cells, conventional methods of plant 5 husbandry and plant breeding can be used to maintain and increase the transformed plants. The transformed plants can also be used in conventional hybridization schemes to produce new plant types which also carry the novel mutant AdT gene (see, e.g., Fehr and Hadley, *Hybridization of Crop Plants* (1980); Jensen, *Plant Breeding Methodology* (1988); Allard, *Principles of Plant Breeding* (1960)).

10 Plants which express a gene which is tolerant or resistant to an inhibitor of AdT can be grown in soil and the herbicide containing the AdT inhibitor can be applied to inhibit weed growth. Since the weed plants will not be carrying the mutant AdT gene, the weeds will be susceptible to the herbicide containing the AdT inhibitor.

15 The following examples are intended to illustrate, but not to limit, aspects of the present invention.

### EXAMPLES

The invention will be further described by reference to the following detailed examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

-40-

Experimental Procedures

**Preparation and purification of recombinant *Bacillus subtilis* GlntRNA<sup>Gln</sup> amidotransferase.** *E. coli* BL21 (DE3) harboring pABC were incubated overnight in 3 mL LB medium (10 g bactotryptone, 5 g yeast extract, 10 g NaCl) with 50 (g/mL 5 ampicillin at 37°C. The culture was scaled up to 1 L and again allowed to incubate at 37°C overnight. Cells were harvested via centrifugation (4000 x g for 5 minutes at 4°C) and resuspended in 20 mL Buffer A (25 mM Hepes-KOH, pH 7.5, 25 mM KCl, 10 mM MgCl<sub>2</sub> and 1 mM DTT). This step and all subsequent steps were performed 10 at 4°C unless otherwise specified. The cells were lysed by sonication (4 x 15 seconds) and centrifuged at 100,000 x g for one hour. The enzyme was then purified to homogeneity, as determined by SDS-polyacrylamide gel electrophoresis, via a series of chromatographic steps using a Pharmacia FPLC system. The supernatant was first applied to a Q-sepharose (HR 16/10) column (strong anion exchange) and the 15 activity was eluted by a linear gradient from 0 to 1 M NaCl in Buffer A. The active fractions from this column were applied to a Superdex-200 (HR 26/100) column (gel filtration) and the activity was eluted isocratically in Buffer A. The fractions from this column which contained activity were pooled and applied onto a MonoQ (HR 20/10) column and the activity as eluted with a linear gradient from 150 to 300 mM NaCl in Buffer A. Active fractions from this column were pooled and dialyzed against Buffer A + 200 mM NaCl in 50% glycerol for 12 hours and stored at 70°C.

**In vivo expressed *Bacillus subtilis* tRNA<sup>Gln</sup> isolation and purification.** A 3 mL culture of *E. coli* DH5a/pGP1-2/pBTT (encoding tRNAGln) in LB medium (10 g bactotryptone, 5 g yeast extract, 10 g NaCl) with 50 (g/mL ampicillin and 10 (g/mL kanamycin was incubated at 37°C overnight. The culture was scaled up to 1 L and overnight incubation was repeated. Cells were harvested via centrifugation at 4000 x g for 5 minutes at 4°C and resuspended in 10 mL lysis buffer (20 mM Tris·HCl, pH 7.4 and 20 mM MgCl<sub>2</sub>). Total nucleic acids were isolated by two sequential extractions with equal volumes of water saturated phenol followed by isopropanol precipitation of the aqueous phase. The nucleic acid pellet was collected via centrifugation at 10,000 x g for 15 minutes at 4°C. The pellet was resuspended in 5 mL of 200 mM Tris·OAc, pH 9.0 and incubated at 37°C for 1 hour to ensure complete deacylation of the tRNA. The nucleic acids were recovered by ethanol precipitation followed by centrifugation at 10,000 x g for 15 minutes at 4°C. The pellet was resuspended in 100 mM NaCl, incubated overnight at 4°C and ethanol precipitated. The tRNAGln was purified by a two-step anion exchange chromatography protocol. The nucleic acids were resuspended in 5 mL of Buffer 1 (140 mM NaOAc, pH 4.5) and 1 gm DE52 resin/100 OD260 was added. The resin was washed with 200 mL Buffer A and 150 mL Buffer 2 (140 mM NaOAc, pH 4.5 + 300 mM NaCl) and the tRNA was eluted with 100 mL Buffer 3 (140 mM NaOAc, pH 4.5 + 1 M NaCl). the nucleic acids were recovered by ethanol precipitation

followed by centrifugation at 10,000 x g for 15 minutes at 4°C and resuspended in 10 mM Tris-HCl, pH 7.4, 1 mM MgCl<sub>2</sub> and 1 mM DTT and applied onto a Pharmacia MonoQ (HR 10/10) column. The tRNA was eluted with a gradient of 450 to 750 mM NaCl in 10 mM Tris-HCl, pH 7.4, 1 mM MgCl<sub>2</sub> and 1 mM DTT. Fractions 5 containing the tRNAGln, based on ability to be aminoacylated with both Glu and Gln, were pooled and used as substrates in the amidotransferase assays.

**Aminoacylation reactions.** The procedure for the formation of radiolabelled GlntRNA<sup>Gln</sup> was adapted from Jahn, D. et al. (1990). Unless otherwise noted, these reactions were conducted at 37°C in a buffer consisting of 10 mM ATP, 50 mM Hepes-KOH pH 7.0, 25 mM KCl, 15 mM MgCl<sub>2</sub>, and 5 mM DTT. The concentration 10 of tRNAGln, recovered from *E. coli* DH5a harboring the plasmids pGP12 and pBTT (see above), and L14C(U)-glutamate (300 mCi/mMol) was 10 (M. GluRS was isolated from *B. subtilis* and then partially purified by DEAE-sepharose chromatography. The reactions were allowed to progress for various lengths of time 15 depending upon the assay. Aliquots from this mixture were then added to the amidotransferase assay mixtures either directly or following water saturated phenol extraction, ethanol precipitation, and resuspension in the aminoacylation buffer.

**Amidotransferase reactions.** The procedure for the formation of radiolabelled GlntRNA<sup>Gln</sup> from GlntRNA<sup>Gln</sup> was adapted from Jahn, D. et al. (1990). 20 Unless otherwise noted, these reactions were conducted at 37°C in a buffer consisting

-43-

of 1 mM ATP, 5 mM Hepes-KOH pH 7.0, 2.5 mM KCl, 1.5 mM MgCl<sub>2</sub>, and 0.5 mM dithiothreitol (DTT). The concentration of L14C(U)-GlutRNA<sup>Gln</sup> was 1 (M and the concentration of Lglutamine was 1 mM. Aliquots (0 to 20 (L) from fractions obtained during purification of the enzyme were added and the mixture was incubated for 5 various lengths of time depending upon the assay followed by quenching with 10 (L, 3 M NaOAc, pH 5.0. The mixture was extracted with an equal volume of water-saturated phenol and the aqueous and organic phases were separated by centrifugation at 15,000 x g at room temperature for 60 seconds. The aqueous phase was removed, 3x volumes of ethanol were added and the tRNA was precipitated at 10 70°C for 15 minutes. The precipitated tRNA was recovered by centrifugation at 15,000 x g at 4°C for 15 minutes. The pellet obtained was resuspended in 50 (L 0.01 N KOH and deaminoacylated at 65°C for 10 minutes. The base was neutralized with 1.3 (L, 0.1 N HCl (to pH ( 6 to 7) and the solution was dried completely under 15 vacuum. The dried pellet was resuspended in 3 (L double-distilled H<sub>2</sub>O and spotted onto a TLC place (cellulose, Aldrich). The front was allowed to migrate 3.5 to 5 hours in one of two solvent systems (A. 20:1:5 isopropyl alcohol:formic acid:water or B. 2:1:6:6 ammonia:water:chloroform:methanol). The plate was dried at 85°C, exposed to an activated phosphoroimaging plate ((12 hours) and the image was analyzed using MacBas v2.0. In this way, the conversion of Glu to Gln was 20 measured.

-44-

### Example 1

#### Characterization of DNA Fragment Encoding AdT

Three genes; one transcript of correct size hybridizing with three probes is provided in Figure 3 (SEQ ID NO:1). Open reading frames for each of the subunits is  
5 provided.

### Example 2

#### Characterization of *B. subtilis* AdT Protein

The amino acid sequence of *B. subtilis* AdT is encoded by the nucleotide sequence of Figure 3 (SEQ ID NO:1).

10 The molecular weights of the three subunits is computed to be: 53.039 Kd, A subunit; 53.314 Kd, B subunit; and 10.859 Kd, C subunit. The amino acid sequences of each of the subunits A, B and C are provided in SEQ ID NOs: 4, 6 and 8, respectively.

15 The sizes of the subunits were confirmed via polyacrylamide gel electrophoresis.

### Example 3

#### Preparation of polyclonal antiserum containing anti-AdT antibodies

A polyclonal antiserum containing anti-AdT antibodies was obtained by administered to rabbits recombinant AdT (trimeric protein) to rabbits following

-45-

known methods. Incubation of the antiserum with a *B. subtilis* extract containing AdT protein completely inhibited AdT activity.

Using polyacrylamide gel electrophoresis, the antiserum was shown to contain antibodies immunoreactive to the assembled AdT protein. The polyclonal antisera 5 was significantly less immunoreactive to non-assembled, individual subunits.

#### Example 4

##### Production and purification of AdT

Table I. Activity of cell extracts from *E. coli* BL21(DE3) harboring various vectors.

10

15

Vector	Glutamine Recovered <i>pMole</i>	Relative Activity
pABC	2.03 ± 0.28	1.000
pA	0.02 ± 0.02	0.010
pB	0.03 ± 0.01	0.015
pC	0.03 ± 0.02	0.017

-46-

**Table II. Purification of *Bacillus subtilis* GlntRNAGln amidotransferase overexpressed in *Escherichia coli* BL21(DE3).**

Purification Step Purification	Total volume mL	Total protein mg	Total activity <sup>a</sup> units x 10 <sup>3</sup>	Specific activity units/mg	Yield %	-fold
S-100	15	190	13.5	70	100	1
Q-sepharoseFF	12	40	8.1	200	60	3
Superdex-200	10	4.6	13.4	3000	99	40
MonoQ	3.5	1.0	8.3	9000	61	130

<sup>a</sup> One unit is defined as 1 pMole glutamine produced per minute at 37°C under the assay conditions described in materials and methods.

### Example 5

## Identifying inhibitors of AdT activity

Purified amidotransferase is used in an assay to identify inhibitors of AdT

15 activity. The assay used to identify inhibitors of AdT activity comprises:

- (a) incubating a first sample of AdT and its substrate;
- (b) measuring an uninhibited reactivity of the AdT from step (a);
- (c) incubating a first sample of AdT and its substrate in the presence of a sample comprising an inhibitor compound;
- (d) measuring an inhibited reactivity of the AdT from step (c); and,

-47-

5 (e) comparing the inhibited reactivity to the uninhibited reactivity of the AdT.

Inhibitors of AdT identified using this process are utilized as antibacterial, antifungal and herbicidal agents.

#### Example 6

##### Identification of inhibitor-resistant AdT mutants

Purified amidotransferase and an identified inhibitor of AdT is used in an assay to identify inhibitor-resistant AdT mutants. The assay used to identify inhibitor-resistant AdT mutants comprises :

10 (a) incubating a first sample of AdT and its substrate in the presence of a second sample comprising an AdT inhibitor;

(b) measuring an unmutated reactivity of the AdT from step (a);

(c) incubating a first sample of a mutated AdT and its substrate in the presence of a second sample comprising an AdT inhibitor;

15 (d) measuring a mutated reactivity of the mutated AdT from step (c); and,

(e) comparing the mutated reactivity to the unmutated reactivity of the AdT.

Inhibitor-resistant AdT mutants identified using this process are utilized in the production of cells and organisms resistant to AdT inhibitors.

**Example 7****Diagnostic assays**

Nucleic acids for diagnosis are obtained from cells or tissues. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. The genomic DNA can be compared to the polynucleotide coding for amidotransferase as provided in SEQ ID NO:1. Deletions and insertions can be detected by a change in size of the amplified product in comparison to SEQ ID NO:1. Point mutations can be identified by hybridizing amplified DNA to labeled AdT polynucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNASE digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in the electrophoretic mobility of the DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or a chemical cleavage method.

Cells carrying mutations or polymorphisms in the gene of the invention may also be detected at the DNA level by a variety of techniques. For example, RTPCR can be used to detect mutations. It is particularly preferred to used RTPCR in conduction with automated detection systems, such as, for example, GeneScan. RNA or cDNA may also be used for the same purpose, PCR or RTPCR. As an example, PCR primers complementary to the nucleic acid encoding AdT can be used to identify

and analyze mutations. These primers may be used for amplifying AdT DNA isolated from a sample derived from an organism. The invention also provides these primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. The primers may be used to amplify the gene isolated from an infected individual such that the gene 5 may then be subject to various techniques for elucidation of the DNA sequence. In this way, mutations in the AdT DNA sequence may be detected and used to diagnose infection and to serotype or classify the infectious agent.

Increased or decreased expression of AdT polynucleotide can be measured using any of the methods well known in the art for the quantitation of polynucleotides, 10 such as, for example, amplification, PCR, RTPCR, RNase protection, Northern blotting and other hybridization methods.

In addition, a diagnostic assay in accordance with the intention for detecting over-or under-expression of AdT protein compared to normal control tissue samples. Assay techniques that can be used to determine levels of AdT protein, in a sample 15 derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassay, competitive-binding assays, Western Blot analysis and ELISA assays.

-50-

### Example 8

#### Production of transformed plants

5        A mutated AdT-encoding DNA sequence that confers resistance to AdT inhibitors is isolated from an inhibitor-resistant AdT mutant using mutagenesis and isolation techniques well known to one of skill in the art. The coding sequence for the mutant AdT gene is then introduced into a plant cell and whole transformed plants are regenerated from the transformed plant cell using a number of different techniques

10      well known to those of skill in the art. The transformed plants are used in conventional plant breeding schemes to produce new varieties of plants which also carry the mutant AdT gene. Crop plants carrying the mutant AdT gene are grown in production and an herbicide comprising an AdT inhibitor is applied to the crop to control weeds.

15      Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. All cited references referred to in the application are hereby incorporated by reference.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: Soll, Dieter

(ii) TITLE OF INVENTION: GLU-TRAN AMIDOTRANSFERASE - A NOVEL  
ESSENTIAL TRANSLATIONAL COMPONENT

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: MORGAN, LEWIS & BOCKIUS LLP
- (B) STREET: 1800 M Street, N.W.
- (C) CITY: Washington
- (D) STATE: D.C.
- (E) COUNTRY: USA
- (F) ZIP: 20036-5869

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: US Unassigned
- (B) FILING DATE: 03-FEB-1998
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 60/037,275
- (B) FILING DATE: 03-FEB-1997

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Adler, Reid G.
- (B) REGISTRATION NUMBER: 30,988
- (C) REFERENCE/DOCKET NUMBER: 044574-5024-WO

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 202-467-7000
- (B) TELEFAX: 202-467-7176

## (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3495 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: join(1..54, 58..390, 394..1866, 1870..3303, 3310  
..3321, 3325..3348, 3352..3429, 3433..3471, 3475  
..3480, 3484..3495)

-52-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAA	TTC	GAT	CCT	GTC	TCA	AGG	CGT	TTT	GTT	GCT	TTA	AAG	GGC	TTG	TTT	48
Glu	Phe	Asp	Pro	Val	Ser	Arg	Arg	Phe	Val	Ala	Leu	Lys	Gly	Leu	Phe	
1	5				10							15				
TTG	ATA	TGA	TCA	GTA	TTA	TAT	GAC	TTA	ACG	GAG	AAA	TAT	GTG	GAG	GTG	96
Leu	Ile			Ser	Val	Leu	Tyr	Asp	Leu	Thr	Glu	Lys	Tyr	Val	Glu	
					20				25			30				
GAT	CAT	ATG	TCA	CGA	ATT	TCA	ATA	GAA	GAA	GTA	AAG	CAC	GTT	GCG	CAC	144
Asp	His	Met	Ser	Arg	Ile	Ser	Ile	Glu	Glu	Val	Lys	His	Val	Ala	His	
					35			40			45					
CTT	GCA	AGA	CTT	GCG	ATT	ACT	GAA	GAA	GCA	AAA	ATG	TTC	ACT	GAA	192	
Leu	Ala	Arg	Leu	Ala	Ile	Thr	Glu	Glu	Glu	Ala	Lys	Met	Phe	Thr	Glu	
					50			55			60					
CAG	CTC	GAC	AGT	ATC	ATT	TCA	TTT	GCC	GAG	GAG	CTT	AAT	GAG	GTT	AAC	240
Gln	Leu	Asp	Ser	Ile	Ile	Ser	Phe	Ala	Glu	Glu	Leu	Asn	Glu	Val	Asn	
					65			70			75					
ACA	GAC	AAT	GTG	GAG	CCT	ACA	ACT	CAC	GTG	CTG	AAA	ATG	AAA	AAT	GTC	288
Thr	Asp	Asn	Val	Glu	Pro	Thr	Thr	His	Val	Leu	Lys	Met	Lys	Asn	Val	
					80			85			90			95		
ATG	AGA	GAA	GAT	GAA	GCG	GGT	AAA	GGT	CTT	CCG	GTT	GAG	GAT	GTC	ATG	336
Met	Arg	Glu	Asp	Glu	Ala	Gly	Lys	Gly	Leu	Pro	Val	Glu	Asp	Val	Met	
					100			105			110					
AAA	AAT	GCG	CCT	GAC	CAT	AAA	GAC	GGC	TAT	ATT	CGT	GTG	CCA	TCA	ATT	384
Lys	Asn	Ala	Pro	Asp	His	Lys	Asp	Gly	Tyr	Ile	Arg	Val	Pro	Ser	Ile	
					115			120			125					
CTG	GAC	TAA	AGG	AGG	GAC	ACA	AGA	ATG	TCA	TTA	TTT	GAT	CAT	AAA	ATC	432
Leu	Asp		Arg	Arg	Asp	Thr	Arg	Met	Ser	Leu	Phe	Asp	His	Lys	Ile	
					130			135			140					
ACA	GAA	TTA	AAA	CAG	CTC	ATA	CAT	AAA	AAA	GAG	ATT	AAG	ATT	TCT	GAT	480
Thr	Glu	Leu	Lys	Gln	Leu	Ile	His	Lys	Lys	Glu	Ile	Lys	Ile	Ser	Asp	
					145			150			155					
CTG	GTT	GAT	GAA	TCT	TAT	AAA	CGC	ATC	CAA	GCG	GTT	GAT	GAT	AAG	GTA	528
Leu	Val	Asp	Glu	Ser	Tyr	Lys	Arg	Ile	Gln	Ala	Val	Asp	Asp	Lys	Val	
					160			165			170					
CAA	GCC	TTT	TTG	GCA	TTA	GAT	GAA	GAA	AGA	GCG	CGC	GCA	TAC	GCG	AAG	576
Gln	Ala	Phe	Leu	Ala	Leu	Asp	Glu	Glu	Arg	Ala	Arg	Ala	Tyr	Ala	Lys	
					175			180			185			190		
GAG	CTT	GAT	GAG	GCG	GTT	GAC	GGC	CGT	TCT	GAG	CAC	GGT	CTT	CTT	TTC	624
Glu	Leu	Asp	Glu	Ala	Val	Asp	Gly	Arg	Ser	Glu	His	Gly	Leu	Leu	Phe	
					195			200			205					
GGT	ATG	CCG	ATC	GGC	GTA	AAA	GAT	AAT	ATC	GTA	ACA	AAA	GGG	CTG	CGC	672
Gly	Met	Pro	Ile	Gly	Val	Lys	Asp	Asn	Ile	Val	Thr	Lys	Gly	Leu	Arg	
					210			215			220					

ACA ACA TGC TCC AGC AAA ATT CTC GAA AAC TTT GAT CCG ATT TAC GAT	720
Thr Thr Cys Ser Ser Lys Ile Leu Glu Asn Phe Asp Pro Ile Tyr Asp	
225 230 235	
GCT ACT GTC GTT CAG CGC CTT CAA GAC GCT GAA GCG GTC ACA ATC GGA	768
Ala Thr Val Val Gln Arg Leu Gln Asp Ala Glu Ala Val Thr Ile Gly	
240 245 250	
AAA CTG AAC ATG GAC GAA TTC GCC ATG GGG TCA TCT ACA GAA AAC TCA	816
Lys Leu Asn Met Asp Glu Phe Ala Met Gly Ser Ser Thr Glu Asn Ser	
255 260 265 270	
GCT TAC AAG CTG ACG AAA AAC CCT TGG AAC CTG GAT ACA GTT CCC GGC	864
Ala Tyr Lys Leu Thr Lys Asn Pro Trp Asn Leu Asp Thr Val Pro Gly	
275 280 285	
GGT TCA AGC GGC GGA TCT GCA GCT GCG GTT GCT GCG GGA GAA GTT CCG	912
Gly Ser Ser Gly Gly Ser Ala Ala Ala Val Ala Ala Gly Glu Val Pro	
290 295 300	
TTT TCT CTT GGA TCT GAC ACA GGC GGC TCC ATC CGT CAG CCG GCA TCT	960
Phe Ser Leu Gly Ser Asp Thr Gly Gly Ser Ile Arg Gln Pro Ala Ser	
305 310 315	
TTC TGC GGC GTT GTC GGA TTA AAA CCT ACA TAC GGA CGT GTA TCT CGT	1008
Phe Cys Gly Val Val Gly Leu Lys Pro Thr Tyr Gly Arg Val Ser Arg	
320 325 330	
TAC GGC CTG GTC GCA TTT GCG TCT TCA TTG GAC CAA ATC GGA CCG ATT	1056
Tyr Gly Leu Val Ala Phe Ala Ser Ser Leu Asp Gln Ile Gly Pro Ile	
335 340 345 350	
ACA CGT ACG GTT GAG GAT AAC GCG TTT TTA CTT CAA GCG ATT TCC GGC	1104
Thr Arg Thr Val Glu Asp Asn Ala Phe Leu Leu Gln Ala Ile Ser Gly	
355 360 365	
GTA GAC AAA ATG GAC TCT ACG AGT GCA AAT GTG GAC GTG CCT GAT TTT	1152
Val Asp Lys Met Asp Ser Thr Ser Ala Asn Val Asp Val Pro Asp Phe	
370 375 380	
CTT TCT TCA TTA ACT GGC GAC ATC AAA GGA CTG AAA ATC GCC GTT CCG	1200
Leu Ser Ser Leu Thr Gly Asp Ile Lys Gly Leu Lys Ile Ala Val Pro	
385 390 395	
AAA GAA TAC CTT GGT GAA GGT GTC GGC AAA GAA GCG AGA GAA TCT GTC	1248
Lys Glu Tyr Leu Gly Glu Gly Val Gly Lys Glu Ala Arg Glu Ser Val	
400 405 410	
TTG GCA GCG CTG AAA GTC CTT GAA GGT CTC GGC GCT ACA TGG GAA GAA	1296
Leu Ala Ala Leu Lys Val Leu Glu Gly Leu Gly Ala Thr Trp Glu Glu	
415 420 425 430	
G TG TCT CTT CCG CAC AGT AAA TAC GCG CTT GCG ACA TAT TAC CTG CTG	1344
Val Ser Leu Pro His Ser Lys Tyr Ala Leu Ala Thr Tyr Tyr Leu Leu	
435 440 445	
TCA TCT TCT GAA GCG TCA GCG AAC CTT GCA CGC TTT GAC GGC ATC CGC	1392
Ser Ser Ser Glu Ala Ser Ala Asn Leu Ala Arg Phe Asp Gly Ile Arg	
450 455 460	

-54-

TAC GGC TAC CGC ACA GCA AAC GCG GAT AAC CTG ATC GAC CTT TAC AAG	1440
Tyr Gly Tyr Arg Thr Asp Asn Ala Asp Asn Leu Ile Asp Leu Tyr Lys	
465 470 475	
CAA ACG CGC GCT GAA GGT TTC GGA AAT GAA GTC AAA CGC CGC ATC ATG	1488
Gln Thr Arg Ala Glu Gly Phe Gly Asn Glu Val Lys Arg Arg Ile Met	
480 485 490	
CTC GGA ACG TTT GCT TTA AGC TCA GGC TAC TAC GAT GCG TAC TAC AAA	1536
Leu Gly Thr Phe Ala Leu Ser Ser Gly Tyr Tyr Asp Ala Tyr Tyr Lys	
495 500 505 510	
AAA GCG CAA AAA GTG CGT ACG TTG ATT AAG AAG GAT TTC GAG GAC GTA	1584
Lys Ala Gln Lys Val Arg Thr Leu Ile Lys Lys Asp Phe Glu Asp Val	
515 520 525	
TTT GAA AAA TAT GAT GTT ATT GTT GGA CCG ACT ACA CCG ACA CCT GCG	1632
Phe Glu Lys Tyr Asp Val Ile Val Gly Pro Thr Thr Pro Thr Pro Ala	
530 535 540	
TTT AAA ATC GGT GAA AAC ACG AAG GAT CCG CTC ACA ATG TAC GCA AAC	1680
Phe Lys Ile Gly Glu Asn Thr Lys Asp Pro Leu Thr Met Tyr Ala Asn	
545 550 555	
GAT ATC TTA ACG ATT CCG GTC AAC CTT GCG GCG TAC CGG GAA TCA GGT	1728
Asp Ile Leu Thr Ile Pro Val Asn Leu Ala Ala Tyr Arg Glu Ser Gly	
560 565 570	
GCC ATG CGG TTA GCA GAC GGA CTT CCG CTC GGC CTG CAA ATC ATC GGA	1776
Ala Met Arg Leu Ala Asp Gly Leu Pro Leu Gly Leu Gln Ile Ile Gly	
575 580 585 590	
AAA CAC TTT GAT GAA GCA CTG TAT ACC GCG TTG CTC ATG CAT TTG AAC	1824
Lys His Phe Asp Glu Ala Leu Tyr Thr Ala Leu Leu Met His Leu Asn	
595 600 605	
AAG CAA CAG ACC ATC ATA AAG CAA AAC CTG AAC TGT AAG GGG	1866
Lys Gln Gln Thr Ile Ile Lys Gln Asn Leu Asn Cys Lys Gly	
610 615 620	
TGA AAA GAA TTG AAC TTT GAA ACG GTA ATC GGA CTT GAA GTC CAC GTT	1914
Lys Glu Leu Asn Phe Glu Thr Val Ile Gly Leu Glu Val His Val	
625 630 635	
GAG TTA AAA ACA AAA TCA AAA ATT TTC TCA AGC TCT CCA ACG CCA TTC	1962
Glu Leu Lys Thr Lys Ser Lys Ile Phe Ser Ser Ser Pro Thr Pro Phe	
640 645 650	
GGC GCG GAG GCG AAT ACG CAG ACA AGC GTT ATT GAC CTC GGA TAT CCG	2010
Gly Ala Glu Ala Asn Thr Gln Thr Ser Val Ile Asp Leu Gly Tyr Pro	
655 660 665	
GGC GTC CTG CCT GTT CTG AAC AAA GAA GCC GTT GAA TTC GCA ATG AAA	2058
Gly Val Leu Pro Val Leu Asn Lys Glu Ala Val Glu Phe Ala Met Lys	
670 675 680	
GCC GCT ATG GCG CTC AAC TGT GAG ATC GCA ACG GAT ACG AAG TTT GAC	2106
Ala Ala Met Ala Leu Asn Cys Glu Ile Ala Thr Asp Thr Lys Phe Asp	
685 690 695	

CGC AAA AAC TAT TTC TAT CCT GAC AAC CCG AAA GCG TAT CAG ATT TCT	2154
Arg Lys Asn Tyr Phe Tyr Pro Asp Asn Pro Lys Ala Tyr Gln Ile Ser	
700 705 710 715	
CAA TTT GAT AAG CCA ATC GGC GAA AAC GGC TGG ATC GAA ATT GAA GTC	2202
Gln Phe Asp Lys Pro Ile Gly Glu Asn Gly Trp Ile Glu Ile Glu Val	
720 725 730	
GGC GGC AAA ACA AAA CGC ATC GGC ATC ACG CGC CTT CAT CTT GAA GAG	2250
Gly Gly Lys Thr Lys Arg Ile Gly Ile Thr Arg Leu His Leu Glu Glu	
735 740 745	
GAT GCC GGA AAA CTG ACG CAT ACG GGC GAC GGC TAT TCT CTT GTT GAC	2298
Asp Ala Gly Lys Leu Thr His Thr Gly Asp Gly Tyr Ser Leu Val Asp	
750 755 760	
TTC AAC CGT CAA GGA ACG CCG CTT GTT GAG TNC GTA TCA GAG CCG GAC	2346
Phe Asn Arg Gln Gly Thr Pro Leu Val Glu Xaa Val Ser Glu Pro Asp	
765 770 775	
ATC CGC ACG CCG GAA GAA NCG TAC GCA TAT CTT GAA AAG CTG AAA TCC	2394
Ile Arg Thr Pro Glu Glu Xaa Tyr Ala Tyr Leu Glu Lys Leu Lys Ser	
780 785 790 795	
ATC ATC CAA TAT ACA GGC GTT TCT GAC TGT AAA ATG GAA GAA GGC TCA	2442
Ile Ile Gln Tyr Thr Gly Val Ser Asp Cys Lys Met Glu Glu Gly Ser	
800 805 810	
CTT CGC TGT GAC GCC AAT ATC TCT CTT CGT CCG ATC GGC CAA GAG GAA	2490
Leu Arg Cys Asp Ala Asn Ile Ser Leu Arg Pro Ile Gly Gln Glu Glu	
815 820 825	
TTC GGC ACA AAA ACA GAA TTG AAA AAC TTG AAC TCC TTT GCG TTT GTT	2538
Phe Gly Thr Lys Thr Glu Leu Lys Asn Leu Asn Ser Phe Ala Phe Val	
830 835 840	
CAA AAA GGC CTT GAG CAT GAA GAA AAA CGC CAG GAG CAG GTT CTT CTT	2586
Gln Lys Gly Leu Glu His Glu Glu Lys Arg Gln Gln Val Leu Leu	
845 850 855	
TCC GGC TTC TTC ATC CAG CAA GAA ACT CGC CGT TAT GAT GAA GCA ACG	2634
Ser Gly Phe Phe Ile Gln Gln Glu Thr Arg Arg Tyr Asp Glu Ala Thr	
860 865 870 875	
AAG AAA ACC ATT CTT ATG CGT GTC AAA GAG GGA TCT GAC GAC TAC CGT	2682
Lys Lys Thr Ile Leu Met Arg Val Lys Glu Gly Ser Asp Asp Tyr Arg	
880 885 890	
TAC TTC CCA GAG CCA GAT CTA GTC GAG CTC TAC ATT GAT GAT GAA TGG	2730
Tyr Phe Pro Glu Pro Asp Leu Val Glu Leu Tyr Ile Asp Asp Glu Trp	
895 900 905	
AAG GAA CGC GTA AAA GCA AGC ATT CCT GAG CTT CCG GAT GAG CGC CGC	2778
Lys Glu Arg Val Lys Ala Ser Ile Pro Glu Leu Pro Asp Glu Arg Arg	
910 915 920	
AAG CGT TAT ATC GAA GAG CTT GGC TTC GCT GCA TAT GAC GCA ATG GTT	2826
Lys Arg Tyr Ile Glu Glu Leu Gly Phe Ala Ala Tyr Asp Ala Met Val	
925 930 935	

CTG ACG CTG ACA AAA GAA ATG GCT GAT TTC TTC GAA GAA ACC GTT CAA	2874
Leu Thr Leu Thr Lys Glu Met Ala Asp Phe Phe Glu Glu Thr Val Gin	
940 945 950 955	
AAA GGC GCT GAA GCT AAA CAA GCG TCT AAC TGG CTG ATG GGT GAA GTG	2922
Lys Gly Ala Glu Ala Lys Gln Ala Ser Asn Trp Leu Met Gly Glu Val	
960 965 970	
TCA GCT TAC CTA AAC GCA GAA CAA AAA GAG CTT GCC GAT GTT GCC CTG	2970
Ser Ala Tyr Leu Asn Ala Glu Gln Lys Glu Leu Ala Asp Val Ala Leu	
975 980 985	
ACA CCT GAA GGC CTT GCC GGC ATG ATC AAA TTG ATT GAA AAA GGA ACC	3018
Thr Pro Glu Gly Leu Ala Gly Met Ile Lys Leu Ile Glu Lys Gly Thr	
990 995 1000	
ATT TCT TCT AAG ATC GCG AAG AAA GTG TTT AAA GAA TTG ATT GAA AAA	3066
Ile Ser Ser Lys Ile Ala Lys Lys Val Phe Lys Glu Leu Ile Glu Lys	
1005 1010 1015	
GGC GGC GAC GCT GAG AAG ATT GTG AAA GAG AAA GGC CTT GTT CAG ATT	3114
Gly Gly Asp Ala Glu Lys Ile Val Lys Glu Lys Gly Leu Val Gln Ile	
1020 1025 1030 1035	
TCT GAC GAA GGC GTG CTT CTG AAG CTT GTC ACT GAG GCG CTT GAC AAC	3162
Ser Asp Glu Gly Val Leu Leu Lys Leu Val Thr Glu Ala Leu Asp Asn	
1040 1045 1050	
AAT CCT CAA TCA ATC GAA GAC TTT AAA AAC GGA AAA GAC CGC GCG ATC	3210
Asn Pro Gln Ser Ile Glu Asp Phe Lys Asn Gly Lys Asp Arg Ala Ile	
1055 1060 1065	
GGC TTC CTA GTC GGA CAG ATT ATG AAA GCG TCC AAA GGA CAA GCC AAC	3258
Gly Phe Leu Val Gly Gln Ile Met Lys Ala Ser Lys Gly Gln Ala Asn	
1070 1075 1080	
CCG CCG ATG GTC AAC AAA ATT CTG CTT GAA GAA ATT AAA AAA CGC	3303
Pro Pro Met Val Asn Lys Ile Leu Leu Glu Glu Ile Lys Lys Arg	
1085 1090 1095	
TAATAA AAA AGC AGC CCT TAG AGG CTG CTT TTT TTA TGG TCA AAT	3348
Lys Ser Ser Pro Arg Leu Leu Phe Leu Trp Ser Asn	
1100 1105 1110	
TGA GAT AAA GAC AAG ATG AGG GCC CGA AGC CTT TCA ACT TCT TTG TCG	3396
Asp Lys Asp Lys Met Arg Ala Arg Ser Leu Ser Thr Ser Leu Ser	
1115 1120 1125	
TTG GTT CCG GCC AAA TTG GAC AGC ATG CCT TTA TAA TCG GCT TGC GCG	3444
Leu Val Pro Ala Lys Leu Asp Ser Met Pro Leu Ser Ala Cys Ala	
1130 1135 1140	
GTT TAT CCT GAG TCA ATT CTT CCT CGA TAA GAT AAG TGA CAC GGT GAT	3492
Val Tyr Pro Glu Ser Ile Leu Pro Arg Asp Lys His Gly Asp	
1145 1150	
ATC	3495
Ile	
1155	

## (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1155 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu	Phe	Asp	Pro	Val	Ser	Arg	Arg	Phe	Val	Ala	Leu	Lys	Gly	Leu	Phe
1				5					10					15	
Leu	Ile	Ser	Val	Leu	Tyr	Asp	Leu	Thr	Glu	Lys	Tyr	Val	Glu	Val	Asp
				20				25				30			
His	Met	Ser	Arg	Ile	Ser	Ile	Glu	Glu	Val	Lys	His	Val	Ala	His	Leu
				35			40				45				
Ala	Arg	Leu	Ala	Ile	Thr	Glu	Glu	Glu	Ala	Lys	Met	Phe	Thr	Glu	Gln
				50		55			60						
Leu	Asp	Ser	Ile	Ile	Ser	Phe	Ala	Glu	Glu	Leu	Asn	Glu	Val	Asn	Thr
				65		70		75			80				
Asp	Asn	Val	Glu	Pro	Thr	Thr	His	Val	Leu	Lys	Met	Lys	Asn	Val	Met
				85			90				95				
Arg	Glu	Asp	Glu	Ala	Gly	Lys	Gly	Leu	Pro	Val	Glu	Asp	Val	Met	Lys
				100			105				110				
Asn	Ala	Pro	Asp	His	Lys	Asp	Gly	Tyr	Ile	Arg	Val	Pro	Ser	Ile	Leu
				115			120			125					
Asp	Arg	Arg	Asp	Thr	Arg	Met	Ser	Leu	Phe	Asp	His	Lys	Ile	Thr	Glu
				130		135			140						
Leu	Lys	Gln	Leu	Ile	His	Lys	Lys	Glu	Ile	Lys	Ile	Ser	Asp	Leu	Val
				145		150			155			160			
Asp	Glu	Ser	Tyr	Lys	Arg	Ile	Gln	Ala	Val	Asp	Asp	Lys	Val	Gln	Ala
				165			170				175				
Phe	Leu	Ala	Leu	Asp	Glu	Glu	Arg	Ala	Arg	Ala	Tyr	Ala	Lys	Glu	Leu
				180			185				190				
Asp	Glu	Ala	Val	Asp	Gly	Arg	Ser	Glu	His	Gly	Leu	Leu	Phe	Gly	Met
				195		200			205						
Pro	Ile	Gly	Val	Lys	Asp	Asn	Ile	Val	Thr	Lys	Gly	Leu	Arg	Thr	Thr
				210		215			220						
Cys	Ser	Ser	Lys	Ile	Leu	Glu	Asn	Phe	Asp	Pro	Ile	Tyr	Asp	Ala	Thr
				225		230			235			240			
Val	Val	Gln	Arg	Leu	Gln	Asp	Ala	Glu	Ala	Val	Thr	Ile	Gly	Lys	Leu
				245			250			255					
Asn	Met	Asp	Glu	Phe	Ala	Met	Gly	Ser	Ser	Thr	Glu	Asn	Ser	Ala	Tyr
				260			265			270					

Lys Leu Thr Lys Asn Pro Trp Asn Leu Asp Thr Val Pro Gly Gly Ser  
 275 280 285  
 Ser Gly Gly Ser Ala Ala Ala Val Ala Ala Gly Glu Val Pro Phe Ser  
 290 295 300  
 Leu Gly Ser Asp Thr Gly Gly Ser Ile Arg Gln Pro Ala Ser Phe Cys  
 305 310 315 320  
 Gly Val Val Gly Leu Lys Pro Thr Tyr Gly Arg Val Ser Arg Tyr Gly  
 325 330 335  
 Leu Val Ala Phe Ala Ser Ser Leu Asp Gln Ile Gly Pro Ile Thr Arg  
 340 345 350  
 Thr Val Glu Asp Asn Ala Phe Leu Leu Gln Ala Ile Ser Gly Val Asp  
 355 360 365  
 Lys Met Asp Ser Thr Ser Ala Asn Val Asp Val Pro Asp Phe Leu Ser  
 370 375 380  
 Ser Leu Thr Gly Asp Ile Lys Gly Leu Lys Ile Ala Val Pro Lys Glu  
 385 390 395 400  
 Tyr Leu Gly Glu Gly Val Gly Lys Glu Ala Arg Glu Ser Val Leu Ala  
 405 410 415  
 Ala Leu Lys Val Leu Glu Gly Leu Gly Ala Thr Trp Glu Glu Val Ser  
 420 425 430  
 Leu Pro His Ser Lys Tyr Ala Leu Ala Thr Tyr Tyr Leu Leu Ser Ser  
 435 440 445  
 Ser Glu Ala Ser Ala Asn Leu Ala Arg Phe Asp Gly Ile Arg Tyr Gly  
 450 455 460  
 Tyr Arg Thr Asp Asn Ala Asp Asn Leu Ile Asp Leu Tyr Lys Gln Thr  
 465 470 475 480  
 Arg Ala Glu Gly Phe Gly Asn Glu Val Lys Arg Arg Ile Met Leu Gly  
 485 490 495  
 Thr Phe Ala Leu Ser Ser Gly Tyr Tyr Asp Ala Tyr Tyr Lys Lys Ala  
 500 505 510  
 Gln Lys Val Arg Thr Leu Ile Lys Lys Asp Phe Glu Asp Val Phe Glu  
 515 520 525  
 Lys Tyr Asp Val Ile Val Gly Pro Thr Thr Pro Thr Pro Ala Phe Lys  
 530 535 540  
 Ile Gly Glu Asn Thr Lys Asp Pro Leu Thr Met Tyr Ala Asn Asp Ile  
 545 550 555 560  
 Leu Thr Ile Pro Val Asn Leu Ala Ala Tyr Arg Glu Ser Gly Ala Met  
 565 570 575  
 Arg Leu Ala Asp Gly Leu Pro Leu Gly Leu Gln Ile Ile Gly Lys His  
 580 585 590

Phe Asp Glu Ala Leu Tyr Thr Ala Leu Leu Met His Leu Asn Lys Gln  
 595 600 605  
 Gln Thr Ile Ile Lys Gln Asn Leu Asn Cys Lys Gly Lys Glu Leu Asn  
 610 615 620  
 Phe Glu Thr Val Ile Gly Leu Glu Val His Val Glu Leu Lys Thr Lys  
 625 630 635 640  
 Ser Lys Ile Phe Ser Ser Ser Pro Thr Pro Phe Gly Ala Glu Ala Asn  
 645 650 655  
 Thr Gln Thr Ser Val Ile Asp Leu Gly Tyr Pro Gly Val Leu Pro Val  
 660 665 670  
 Leu Asn Lys Glu Ala Val Glu Phe Ala Met Lys Ala Ala Met Ala Leu  
 675 680 685  
 Asn Cys Glu Ile Ala Thr Asp Thr Lys Phe Asp Arg Lys Asn Tyr Phe  
 690 695 700  
 Tyr Pro Asp Asn Pro Lys Ala Tyr Gln Ile Ser Gln Phe Asp Lys Pro  
 705 710 715 720  
 Ile Gly Glu Asn Gly Trp Ile Glu Ile Glu Val Gly Gly Lys Thr Lys  
 725 730 735  
 Arg Ile Gly Ile Thr Arg Leu His Leu Glu Glu Asp Ala Gly Lys Leu  
 740 745 750  
 Thr His Thr Gly Asp Gly Tyr Ser Leu Val Asp Phe Asn Arg Gln Gly  
 755 760 765  
 Thr Pro Leu Val Glu Xaa Val Ser Glu Pro Asp Ile Arg Thr Pro Glu  
 770 775 780  
 Glu Xaa Tyr Ala Tyr Leu Glu Lys Leu Lys Ser Ile Ile Gln Tyr Thr  
 785 790 795 800  
 Gly Val Ser Asp Cys Lys Met Glu Glu Gly Ser Leu Arg Cys Asp Ala  
 805 810 815  
 Asn Ile Ser Leu Arg Pro Ile Gly Gln Glu Glu Phe Gly Thr Lys Thr  
 820 825 830  
 Glu Leu Lys Asn Leu Asn Ser Phe Ala Phe Val Gln Lys Gly Leu Glu  
 835 840 845  
 His Glu Glu Lys Arg Gln Glu Gln Val Leu Leu Ser Gly Phe Phe Ile  
 850 855 860  
 Gln Gln Glu Thr Arg Arg Tyr Asp Glu Ala Thr Lys Lys Thr Ile Leu  
 865 870 875 880  
 Met Arg Val Lys Glu Gly Ser Asp Asp Tyr Arg Tyr Phe Pro Glu Pro  
 885 890 895  
 Asp Leu Val Glu Leu Tyr Ile Asp Asp Glu Trp Lys Glu Arg Val Lys  
 900 905 910

Ala Ser Ile Pro Glu Leu Pro Asp Glu Arg Arg Lys Arg Tyr Ile Glu  
 915 920 925  
 Glu Leu Gly Phe Ala Ala Tyr Asp Ala Met Val Leu Thr Leu Thr Lys  
 930 935 940  
 Glu Met Ala Asp Phe Phe Glu Glu Thr Val Gln Lys Gly Ala Glu Ala  
 945 950 955 960  
 Lys Gln Ala Ser Asn Trp Leu Met Gly Glu Val Ser Ala Tyr Leu Asn  
 965 970 975  
 Ala Glu Gln Lys Glu Leu Ala Asp Val Ala Leu Thr Pro Glu Gly Leu  
 980 985 990  
 Ala Gly Met Ile Lys Leu Ile Glu Lys Gly Thr Ile Ser Ser Lys Ile  
 995 1000 1005  
 Ala Lys Lys Val Phe Lys Glu Leu Ile Glu Lys Gly Gly Asp Ala Glu  
 1010 1015 1020  
 Lys Ile Val Lys Glu Lys Gly Leu Val Gln Ile Ser Asp Glu Gly Val  
 1025 1030 1035 1040  
 Leu Leu Lys Leu Val Thr Glu Ala Leu Asp Asn Asn Pro Gln Ser Ile  
 1045 1050 1055  
 Glu Asp Phe Lys Asn Gly Lys Asp Arg Ala Ile Gly Phe Leu Val Gly  
 1060 1065 1070  
 Gln Ile Met Lys Ala Ser Lys Gly Gln Ala Asn Pro Pro Met Val Asn  
 1075 1080 1085  
 Lys Ile Leu Leu Glu Glu Ile Lys Lys Arg Lys Ser Ser Pro Arg Leu  
 1090 1095 1100  
 Leu Phe Leu Trp Ser Asn Asp Lys Asp Lys Met Arg Ala Arg Ser Leu  
 1105 1110 1115 1120  
 Ser Thr Ser Leu Ser Leu Val Pro Ala Lys Leu Asp Ser Met Pro Leu  
 1125 1130 1135  
 Ser Ala Cys Ala Val Tyr Pro Glu Ser Ile Leu Pro Arg Asp Lys His  
 1140 1145 1150  
 Gly Asp Ile  
 1155

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1461 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
 

- (A) NAME/KEY: CDS

(B) LOCATION: 1..1458

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG TCA TTA TTT GAT CAT AAA ATC ACA GAA TTA AAA CAG CTC ATA CAT	48
Met Ser Leu Phe Asp His Lys Ile Thr Glu Leu Lys Gln Leu Ile His	
1 5 10 15	
AAA AAA GAG ATT AAG ATT TCT GAT CTG GTT GAT GAA TCT TAT AAA CGC	96
Lys Lys Glu Ile Lys Ile Ser Asp Leu Val Asp Glu Ser Tyr Lys Arg	
20 25 30	
ATC CAA GCG GTT GAT GAT AAG GTA CAA GCC TTT TTG GCA TTA GAT GAA	144
Ile Gln Ala Val Asp Asp Lys Val Gln Ala Phe Leu Ala Leu Asp Glu	
35 40 45	
GAA AGA GCG CGC GCA TAC GCG AAG GAG CTT GAT GAG GCG GTT GAC GGC	192
Glu Arg Ala Arg Ala Tyr Ala Lys Glu Leu Asp Glu Ala Val Asp Gly	
50 55 60	
CGT TCT GAG CAC GGT CTT CTT TTC GGT ATG CCG ATC GGC GTA AAA GAT	240
Arg Ser Glu His Gly Leu Leu Phe Gly Met Pro Ile Gly Val Lys Asp	
65 70 75 80	
AAT ATC GTA ACA AAA GGG CTG CGC ACA ACA TGC TCC AGC AAA ATT CTC	288
Asn Ile Val Thr Lys Gly Leu Arg Thr Thr Cys Ser Ser Lys Ile Leu	
85 90 95	
GAA AAC TTT GAT CCG ATT TAC GAT GCT ACT GTC GTT CAG CGC CTT CAA	336
Glu Asn Phe Asp Pro Ile Tyr Asp Ala Thr Val Val Gln Arg Leu Gln	
100 105 110	
GAC GCT GAA GCG GTC ACA ATC GGA AAA CTG AAC ATG GAC GAA TTC GCC	384
Asp Ala Glu Ala Val Thr Ile Gly Lys Leu Asn Met Asp Glu Phe Ala	
115 120 125	
ATG GGG TCA TCT ACA GAA AAC TCA GCT TAC AAG CTG ACG AAA AAC CCT	432
Met Gly Ser Ser Thr Glu Asn Ser Ala Tyr Lys Leu Thr Lys Asn Pro	
130 135 140	
TGG AAC CTG GAT ACA GTT CCC GGC GGT TCA AGC GGC GGA TCT GCA GCT	480
Trp Asn Leu Asp Thr Val Pro Gly Gly Ser Ser Gly Gly Ser Ala Ala	
145 150 155 160	
GCG GTT GCT GCG GGA GAA GTT CCG TTT TCT CTT GGA TCT GAC ACA GGC	528
Ala Val Ala Ala Gly Glu Val Pro Phe Ser Leu Gly Ser Asp Thr Gly	
165 170 175	
GGC TCC ATC CGT CAG CCG GCA TCT TTC TGC GGC GTT GTC GGA TTA AAA	576
Gly Ser Ile Arg Gln Pro Ala Ser Phe Cys Gly Val Val Gly Leu Lys	
180 185 190	
CCT ACA TAC GGA CGT GTA TCT CGT TAC GGC CTG GTC GCA TTT GCG TCT	624
Pro Thr Tyr Gly Arg Val Ser Arg Tyr Gly Leu Val Ala Phe Ala Ser	
195 200 205	
TCA TTG GAC CAA ATC GGA CCG ATT ACA CGT ACG GTT GAG GAT AAC GCG	672
Ser Leu Asp Gln Ile Gly Pro Ile Thr Arg Thr Val Glu Asp Asn Ala	
210 215 220	

TTT TTA CTT CAA GCG ATT TCC GGC GTA GAC AAA ATG GAC TCT ACG AGT Phe Leu Leu Gln Ala Ile Ser Gly Val Asp Lys Met Asp Ser Thr Ser 225 230 235 240	720
GCA AAT GTG GAC GTG CCT GAT TTT CTT TCT TCA TTA ACT GGC GAC ATC Ala Asn Val Asp Val Pro Asp Phe Leu Ser Ser Leu Thr Gly Asp Ile 245 250 255	768
AAA GGA CTG AAA ATC GCC GTT CCG AAA GAA TAC CTT GGT GAA GGT GTC Lys Gly Leu Lys Ile Ala Val Pro Lys Glu Tyr Leu Gly Glu Gly Val 260 265 270	816
GGC AAA GAA GCG AGA GAA TCT GTC TTG GCA GCG CTG AAA GTC CTT GAA Gly Lys Glu Ala Arg Glu Ser Val Leu Ala Ala Leu Lys Val Leu Glu 275 280 285	864
GGT CTC GGC GCT ACA TGG GAA GAA GTG TCT CTT CCG CAC AGT AAA TAC Gly Leu Gly Ala Thr Trp Glu Val Ser Leu Pro His Ser Lys Tyr 290 295 300	912
GCG CTT GCG ACA TAT TAC CTG CTG TCA TCT TCT GAA GCG TCA GCG AAC Ala Leu Ala Thr Tyr Tyr Leu Leu Ser Ser Ser Glu Ala Ser Ala Asn 305 310 315 320	960
CTT GCA CGC TTT GAC GGC ATC CGC TAC GGC TAC CGC ACA GAC AAC GCG Leu Ala Arg Phe Asp Gly Ile Arg Tyr Gly Tyr Arg Thr Asp Asn Ala 325 330 335	1008
GAT AAC CTG ATC GAC CTT TAC AAG CAA ACG CGC GCT GAA GGT TTC GGA Asp Asn Leu Ile Asp Leu Tyr Lys Gln Thr Arg Ala Glu Gly Phe Gly 340 345 350	1056
AAT GAA GTC AAA CGC CGC ATC ATG CTC GGA ACG TTT GCT TTA AGC TCA Asn Glu Val Lys Arg Arg Ile Met Leu Gly Thr Phe Ala Leu Ser Ser 355 360 365	1104
GGC TAC TAC GAT GCG TAC TAC AAA AAA GCG CAA AAA GTG CGT ACG TTG Gly Tyr Tyr Asp Ala Tyr Tyr Lys Lys Ala Gln Lys Val Arg Thr Leu 370 375 380	1152
ATT AAG AAG GAT TTC GAG GAC GTA TTT GAA AAA TAT GAT GTT ATT GTT Ile Lys Lys Asp Phe Glu Asp Val Phe Glu Lys Tyr Asp Val Ile Val 385 390 395 400	1200
GGA CCG ACT ACA CCG ACA CCT GCG TTT AAA ATC GGT GAA AAC ACG AAG Gly Pro Thr Thr Pro Thr Pro Ala Phe Lys Ile Gly Glu Asn Thr Lys 405 410 415	1248
GAT CCG CTC ACA ATG TAC GCA AAC GAT ATC TTA ACG ATT CCG GTC AAC Asp Pro Leu Thr Met Tyr Ala Asn Asp Ile Leu Thr Ile Pro Val Asn 420 425 430	1296
CTT GCG GCG TAC CGG GAA TCA GGT GCC ATG CGG TTA GCA GAC GGA CTT Leu Ala Ala Tyr Arg Glu Ser Gly Ala Met Arg Leu Ala Asp Gly Leu 435 440 445	1344
CCG CTC GGC CTG CAA ATC ATC GGA AAA CAC TTT GAT GAA GCA CTG TAT Pro Leu Gly Leu Gln Ile Ile Gly Lys His Phe Asp Glu Ala Leu Tyr 450 455 460	1392

ACC GCG TTG CTC ATG CAT TTG AAC AAG CAA CAG ACC ATC ATA AAG CAA 1440  
 Thr Ala Leu Leu Met His Leu Asn Lys Gln Gln Thr Ile Ile Lys Gln  
 465 470 475 480

AAC CTG AAC TGT AAG GGG TGA 1461  
 Asn Leu Asn Cys Lys Gly  
 485

## (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 486 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ser Leu Phe Asp His Lys Ile Thr Glu Leu Lys Gln Leu Ile His  
 1 5 10 15

Lys Lys Glu Ile Lys Ile Ser Asp Leu Val Asp Glu Ser Tyr Lys Arg  
 20 25 30

Ile Gin Ala Val Asp Asp Lys Val Gln Ala Phe Leu Ala Leu Asp Glu  
 35 40 45

Glu Arg Ala Arg Ala Tyr Ala Lys Glu Leu Asp Glu Ala Val Asp Gly  
 50 55 60

Arg Ser Glu His Gly Leu Leu Phe Gly Met Pro Ile Gly Val Lys Asp  
 65 70 75 80

Asn Ile Val Thr Lys Gly Leu Arg Thr Thr Cys Ser Ser Lys Ile Leu  
 85 90 95

Glu Asn Phe Asp Pro Ile Tyr Asp Ala Thr Val Val Gln Arg Leu Gln  
 100 105 110

Asp Ala Glu Ala Val Thr Ile Gly Lys Leu Asn Met Asp Glu Phe Ala  
 115 120 125

Met Gly Ser Ser Thr Glu Asn Ser Ala Tyr Lys Leu Thr Lys Asn Pro  
 130 135 140

Trp Asn Leu Asp Thr Val Pro Gly Gly Ser Ser Gly Gly Ser Ala Ala  
 145 150 155 160

Ala Val Ala Ala Gly Glu Val Pro Phe Ser Leu Gly Ser Asp Thr Gly  
 165 170 175

Gly Ser Ile Arg Gln Pro Ala Ser Phe Cys Gly Val Val Gly Leu Lys  
 180 185 190

Pro Thr Tyr Gly Arg Val Ser Arg Tyr Gly Leu Val Ala Phe Ala Ser  
 195 200 205

Ser Leu Asp Gln Ile Gly Pro Ile Thr Arg Thr Val Glu Asp Asn Ala  
 210 215 220

Phe Leu Leu Gln Ala Ile Ser Gly Val Asp Lys Met Asp Ser Thr Ser  
 225 230 235 240  
 Ala Asn Val Asp Val Pro Asp Phe Leu Ser Ser Leu Thr Gly Asp Ile  
 245 250 255  
 Lys Gly Leu Lys Ile Ala Val Pro Lys Glu Tyr Leu Gly Glu Gly Val  
 260 265 270  
 Gly Lys Glu Ala Arg Glu Ser Val Leu Ala Ala Leu Lys Val Leu Glu  
 275 280 285  
 Gly Leu Gly Ala Thr Trp Glu Glu Val Ser Leu Pro His Ser Lys Tyr  
 290 295 300  
 Ala Leu Ala Thr Tyr Tyr Leu Leu Ser Ser Ser Glu Ala Ser Ala Asn  
 305 310 315 320  
 Leu Ala Arg Phe Asp Gly Ile Arg Tyr Gly Tyr Arg Thr Asp Asn Ala  
 325 330 335  
 Asp Asn Leu Ile Asp Leu Tyr Lys Gln Thr Arg Ala Glu Gly Phe Gly  
 340 345 350  
 Asn Glu Val Lys Arg Arg Ile Met Leu Gly Thr Phe Ala Leu Ser Ser  
 355 360 365  
 Gly Tyr Tyr Asp Ala Tyr Tyr Lys Lys Ala Gln Lys Val Arg Thr Leu  
 370 375 380  
 Ile Lys Lys Asp Phe Glu Asp Val Phe Glu Lys Tyr Asp Val Ile Val  
 385 390 395 400  
 Gly Pro Thr Thr Pro Thr Pro Ala Phe Lys Ile Gly Glu Asn Thr Lys  
 405 410 415  
 Asp Pro Leu Thr Met Tyr Ala Asn Asp Ile Leu Thr Ile Pro Val Asn  
 420 425 430  
 Leu Ala Ala Tyr Arg Glu Ser Gly Ala Met Arg Leu Ala Asp Gly Leu  
 435 440 445  
 Pro Leu Gly Leu Gln Ile Ile Gly Lys His Phe Asp Glu Ala Leu Tyr  
 450 455 460  
 Thr Ala Leu Leu Met His Leu Asn Lys Gln Gln Thr Ile Ile Lys Gln  
 465 470 475 480  
 Asn Leu Asn Cys Lys Gly  
 485

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1431 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

-65-

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1428

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTG AAC TTT GAA ACG GTA ATC GGA CTT GAA GTC CAC GTT GAG TTA AAA	48
Leu Asn Phe Glu Thr Val Ile Gly Leu Glu Val His Val Glu Leu Lys	
1 5 10 15	
ACA AAA TCA AAA ATT TTC TCA AGC TCT CCA ACG CCA TTC GGC GCG GAG	96
Thr Lys Ser Lys Ile Phe Ser Ser Ser Pro Thr Pro Phe Gly Ala Glu	
20 25 30	
GCG AAT ACG CAG ACA AGC GTT ATT GAC CTC GGA TAT CCG GGC GTC CTG	144
Ala Asn Thr Gln Thr Ser Val Ile Asp Leu Gly Tyr Pro Gly Val Leu	
35 40 45	
CCT GTT CTG AAC AAA GAA GCC GTT GAA TTC GCA ATG AAA GCC GCT ATG	192
Pro Val Leu Asn Lys Glu Ala Val Glu Phe Ala Met Lys Ala Ala Met	
50 55 60	
GCG CTC AAC TGT GAG ATC GCA ACG GAT ACG AAG TTT GAC CGC AAA AAC	240
Ala Leu Asn Cys Glu Ile Ala Thr Asp Thr Lys Phe Asp Arg Lys Asn	
65 70 75 80	
TAT TTC TAT CCT GAC AAC CCG AAA GCG TAT CAG ATT TCT CAA TTT GAT	288
Tyr Phe Tyr Pro Asp Asn Pro Lys Ala Tyr Gln Ile Ser Gln Phe Asp	
85 90 95	
AAG CCA ATC GGC GAA AAC GGC TGG ATC GAA ATT GAA GTC GGC GGC AAA	336
Lys Pro Ile Gly Glu Asn Gly Trp Ile Glu Ile Glu Val Gly Gly Lys	
100 105 110	
ACA AAA CGC ATC GGC ATC ACG CGC CTT CAT CTT GAA GAG GAT GCC GGA	384
Thr Lys Arg Ile Gly Ile Thr Arg Leu His Leu Glu Glu Asp Ala Gly	
115 120 125	
AAA CTG ACG CAT ACG GGC GAC GGC TAT TCT CTT GTT GAC TTC AAC CGT	432
Lys Leu Thr His Thr Gly Asp Gly Tyr Ser Leu Val Asp Phe Asn Arg	
130 135 140	
CAA GGA ACG CCG CTT GTT GAG TNC GTA TCA GAG CCG GAC ATC CGC ACG	480
Gln Gly Thr Pro Leu Val Glu Xaa Val Ser Glu Pro Asp Ile Arg Thr	
145 150 155 160	
CCG GAA GAA NCG TAC GCA TAT CTT GAA AAG CTG AAA TCC ATC ATC CAA	528
Pro Glu Glu Xaa Tyr Ala Tyr Leu Glu Lys Leu Lys Ser Ile Ile Gln	
165 170 175	
TAT ACA GGC GTT TCT GAC TGT AAA ATG GAA GAA GGC TCA CTT CGC TGT	576
Tyr Thr Gly Val Ser Asp Cys Lys Met Glu Glu Gly Ser Leu Arg Cys	
180 185 190	
GAC GCC AAT ATC TCT CTT CGT CCG ATC GGC CAA GAG GAA TTC GGC ACA	624
Asp Ala Asn Ile Ser Leu Arg Pro Ile Gly Gln Glu Glu Phe Gly Thr	
195 200 205	

AAA ACA GAA TTG AAA AAC TTG AAC TCC TTT GCG TTT GTT CAA AAA GGC Lys Thr Glu Leu Lys Asn Leu Asn Ser Phe Ala Phe Val Gln Lys Gly 210 215 220	672
CTT GAG CAT GAA GAA AAA CGC CAG GAG CAG GTT CTT CTT TCC GGC TTC Leu Glu His Glu Glu Lys Arg Gln Glu Gln Val Leu Leu Ser Gly Phe 225 230 235 240	720
TTC ATC CAG CAA GAA ACT CGC CGT TAT GAT GAA GCA ACG AAG AAA ACC Phe Ile Gln Gln Glu Thr Arg Arg Tyr Asp Glu Ala Thr Lys Thr 245 250 255	768
ATT CTT ATG CGT GTC AAA GAG GGA TCT GAC GAC TAC CGT TAC TTC CCA Ile Leu Met Arg Val Lys Glu Gly Ser Asp Asp Tyr Arg Tyr Phe Pro 260 265 270	816
GAG CCA GAT CTA GTC GAG CTC TAC ATT GAT GAT GAA TGG AAG GAA CGC Glu Pro Asp Leu Val Glu Leu Tyr Ile Asp Asp Glu Trp Lys Glu Arg 275 280 285	864
GTA AAA GCA AGC ATT CCT GAG CTT CCG GAT GAG CGC CGC AAG CGT TAT Val Lys Ala Ser Ile Pro Glu Leu Pro Asp Glu Arg Arg Lys Arg Tyr 290 295 300	912
ATC GAA GAG CTT GGC TTC GCT GCA TAT GAC GCA ATG GTT CTG ACG CTG Ile Glu Glu Leu Gly Phe Ala Ala Tyr Asp Ala Met Val Leu Thr Leu 305 310 315 320	960
ACA AAA GAA ATG GCT GAT TTC TTC GAA GAA ACC GTT CAA AAA GGC GCT Thr Lys Glu Met Ala Asp Phe Glu Glu Thr Val Gln Lys Gly Ala 325 330 335	1008
GAA GCT AAA CAA GCG TCT AAC TGG CTG ATG GGT GAA GTG TCA GCT TAC Glu Ala Lys Gln Ala Ser Asn Trp Leu Met Gly Glu Val Ser Ala Tyr 340 345 350	1056
CTA AAC GCA GAA CAA AAA GAG CTT GCC GAT GTT GCC CTG ACA CCT GAA Leu Asn Ala Glu Gln Lys Glu Leu Ala Asp Val Ala Leu Thr Pro Glu 355 360 365	1104
GGC CTT GCC GGC ATG ATC AAA TTG ATT GAA AAA GGA ACC ATT TCT TCT Gly Leu Ala Gly Met Ile Lys Leu Ile Glu Lys Gly Thr Ile Ser Ser 370 375 380	1152
AAG ATC GCG AAG AAA GTG TTT AAA GAA TTG ATT GAA AAA GGC GGC GAC Lys Ile Ala Lys Lys Val Phe Lys Glu Leu Ile Glu Lys Gly Gly Asp 385 390 395 400	1200
GCT GAG AAG ATT GTG AAA GAG AAA GGC CTT GTT CAG ATT TCT GAC GAA Ala Glu Lys Ile Val Lys Glu Lys Gly Leu Val Gln Ile Ser Asp Glu 405 410 415	1248
GGC GTG CTT CTG AAG CTT GTC ACT GAG GCG CTT GAC AAC AAT CCT CAA Gly Val Leu Leu Lys Leu Val Thr Glu Ala Leu Asp Asn Asn Pro Gln 420 425 430	1296
TCA ATC GAA GAC TTT AAA AAC GGA AAA GAC CGC GCG ATC GGC TTC CTA Ser Ile Glu Asp Phe Lys Asn Gly Lys Asp Arg Ala Ile Gly Phe Leu 435 440 445	1344

GTC GGA CAG ATT ATG AAA GCG TCC AAA GGA CAA GCC AAC CCG CCG ATG 1392  
 Val Gly Gln Ile Met Lys Ala Ser Lys Gly Gln Ala Asn Pro Pro Met  
 450 455 460

GTC AAC AAA ATT CTG CTT GAA GAA ATT AAA AAA CGC TAA 1431  
 Val Asn Lys Ile Leu Leu Glu Glu Ile Lys Lys Arg  
 465 470 475

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 476 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Asn Phe Glu Thr Val Ile Gly Leu Glu Val His Val Glu Leu Lys  
 1 5 10 15

Thr Lys Ser Lys Ile Phe Ser Ser Ser Pro Thr Pro Phe Gly Ala Glu  
 20 25 30

Ala Asn Thr Gln Thr Ser Val Ile Asp Leu Gly Tyr Pro Gly Val Leu  
 35 40 45

Pro Val Leu Asn Lys Glu Ala Val Glu Phe Ala Met Lys Ala Ala Met  
 50 55 60

Ala Leu Asn Cys Glu Ile Ala Thr Asp Thr Lys Phe Asp Arg Lys Asn  
 65 70 75 80

Tyr Phe Tyr Pro Asp Asn Pro Lys Ala Tyr Gln Ile Ser Gln Phe Asp  
 85 90 95

Lys Pro Ile Gly Glu Asn Gly Trp Ile Glu Ile Glu Val Gly Gly Lys  
 100 105 110

Thr Lys Arg Ile Gly Ile Thr Arg Leu His Leu Glu Glu Asp Ala Gly  
 115 120 125

Lys Leu Thr His Thr Gly Asp Gly Tyr Ser Leu Val Asp Phe Asn Arg  
 130 135 140

Gln Gly Thr Pro Leu Val Glu Xaa Val Ser Glu Pro Asp Ile Arg Thr  
 145 150 155 160

Pro Glu Glu Xaa Tyr Ala Tyr Leu Glu Lys Leu Lys Ser Ile Ile Gln  
 165 170 175

Tyr Thr Gly Val Ser Asp Cys Lys Met Glu Glu Gly Ser Leu Arg Cys  
 180 185 190

Asp Ala Asn Ile Ser Leu Arg Pro Ile Gly Gln Glu Glu Phe Gly Thr  
 195 200 205

Lys Thr Glu Leu Lys Asn Leu Asn Ser Phe Ala Phe Val Gln Lys Gly  
 210 215 220

Leu Glu His Glu Glu Lys Arg Gln Glu Gln Val Leu Leu Ser Gly Phe  
225 230 235 240

Phe Ile Gln Gln Glu Thr Arg Arg Tyr Asp Glu Ala Thr Lys Lys Thr  
245 250 255

Ile Leu Met Arg Val Lys Glu Gly Ser Asp Asp Tyr Arg Tyr Phe Pro  
260 265 270

Glu Pro Asp Leu Val Glu Leu Tyr Ile Asp Asp Glu Trp Lys Glu Arg  
275 280 285

Val Lys Ala Ser Ile Pro Glu Leu Pro Asp Glu Arg Arg Lys Arg Tyr  
290 295 300

Ile Glu Glu Leu Gly Phe Ala Ala Tyr Asp Ala Met Val Leu Thr Leu  
305 310 315 320

Thr Lys Glu Met Ala Asp Phe Phe Glu Glu Thr Val Gln Lys Gly Ala  
325 330 335

Glu Ala Lys Gln Ala Ser Asn Trp Leu Met Gly Glu Val Ser Ala Tyr  
340 345 350

Leu Asn Ala Glu Gln Lys Glu Leu Ala Asp Val Ala Leu Thr Pro Glu  
355 360 365

Gly Leu Ala Gly Met Ile Lys Leu Ile Glu Lys Gly Thr Ile Ser Ser  
370 375 380

Lys Ile Ala Lys Lys Val Phe Lys Glu Leu Ile Glu Lys Gly Gly Asp  
385 390 395 400

Ala Glu Lys Ile Val Lys Glu Lys Gly Leu Val Gln Ile Ser Asp Glu  
405 410 415

Gly Val Leu Leu Lys Leu Val Thr Glu Ala Leu Asp Asn Asn Pro Gln  
420 425 430

Ser Ile Glu Asp Phe Lys Asn Gly Lys Asp Arg Ala Ile Gly Phe Leu  
435 440 445

Val Gly Gln Ile Met Lys Ala Ser Lys Gly Gln Ala Asn Pro Pro Met  
450 455 460

Val Asn Lys Ile Leu Leu Glu Glu Ile Lys Lys Arg  
465 470 475

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 291 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:

-69-

(A) NAME/KEY: CDS  
 (B) LOCATION: 1..288

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG TCA CGA ATT TCA ATA GAA GAA GTA AAG CAC GTT GCG CAC CTT GCA Met Ser Arg Ile Ser Ile Glu Glu Val Lys His Val Ala His Leu Ala	48
1 5 10 15	
AGA CTT GCG ATT ACT GAA GAA GCA AAA ATG TTC ACT GAA CAG CTC Arg Leu Ala Ile Thr Glu Glu Ala Lys Met Phe Thr Glu Gln Leu	96
20 25 30	
GAC AGT ATC ATT TCA TTT GCC GAG GAG CTT AAT GAG GTT AAC ACA GAC Asp Ser Ile Ile Ser Phe Ala Glu Glu Leu Asn Glu Val Asn Thr Asp	144
35 40 45	
AAT GTG GAG CCT ACA ACT CAC GTG CTG AAA ATG AAA AAT GTC ATG AGA Asn Val Glu Pro Thr Thr His Val Leu Lys Met Lys Asn Val Met Arg	192
50 55 60	
GAA GAT GAA GCG GGT AAA GGT CTT CCG GTT GAG GAT GTC ATG AAA AAT Glu Asp Glu Ala Gly Lys Gly Leu Pro Val Glu Asp Val Met Lys Asn	240
65 70 75 80	
GCG CCT GAC CAT AAA GAC GGC TAT ATT CGT GTG CCA TCA ATT CTG GAC Ala Pro Asp His Lys Asp Gly Tyr Ile Arg Val Pro Ser Ile Leu Asp	288
85 90 95	
TAA	291

## (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 96 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ser Arg Ile Ser Ile Glu Glu Val Lys His Val Ala His Leu Ala 1 5 10 15	
Arg Leu Ala Ile Thr Glu Glu Ala Lys Met Phe Thr Glu Gln Leu 20 25 30	
Asp Ser Ile Ile Ser Phe Ala Glu Glu Leu Asn Glu Val Asn Thr Asp 35 40 45	
Asn Val Glu Pro Thr Thr His Val Leu Lys Met Lys Asn Val Met Arg 50 55 60	
Glu Asp Glu Ala Gly Lys Gly Leu Pro Val Glu Asp Val Met Lys Asn 65 70 75 80	
Ala Pro Asp His Lys Asp Gly Tyr Ile Arg Val Pro Ser Ile Leu Asp 85 90 95	

-70-

Claims

1. An isolated polynucleotide encoding an amidotransferase (AdT) protein.
2. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
  - (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide encoded by SEQ ID NO:1;
  - (b) a polynucleotide which is complementary to the polynucleotide of (a); and,
  - (c) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a) or (b).
3. The polynucleotide of claim 2 wherein the polynucleotide is DNA.
4. The polynucleotide of claim 2 wherein the polynucleotide is RNA.
5. The polynucleotide of claim 3 comprising the nucleotides 103 to 3306 set forth in SEQ ID NO:1.
6. A vector comprising the DNA of claim 3.
7. A host cell comprising the vector of claim 6.

-71-

8. A process for producing a polypeptide comprising expressing from the host cell of claim 7 a polypeptide encoded by said DNA.
9. A process for producing a cell which expresses a polypeptide comprising transforming or transfecting the cell with the vector of claim 6 such that the cell expresses the polypeptide encoded by the DNA contained in the vector.
10. A process for producing an amidotransferase polypeptide or amidotransferase fragment comprising culturing a host cell of claim 7 under conditions sufficient for the production of said polypeptide or fragment.
11. A polypeptide comprising an amino acid sequence which is at least 70% identical to a polypeptide encoded by nucleotides 103 to 3306 set forth in SEQ ID NO:1.
12. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 11.
13. An antagonist which inhibits the activity of the polypeptide of claim 11.

-72-

14. A method for the treatment of an individual having need of amidotransferase comprising administering to the individual a therapeutically effective amount of the polypeptide of claim 11.
15. The method of claim 14 wherein said therapeutically effective amount of the polypeptide is administered by providing to the individual DNA encoding said polypeptide and expressing said polypeptide *in vivo*.
16. A method for the treatment of an individual having need to inhibit amidotransferase polypeptide comprising administering to the individual a therapeutically effective amount of the antagonist of claim 13.
17. A process for diagnosing a disease related to expression of the polypeptide of claim 11 comprising determining a nucleic acid sequence encoding said polypeptide.
18. A diagnostic process comprising analyzing for the presence of the polypeptide of claim 11 in a sample derived from a host.
19. A method for identifying compounds which bind to and inhibit an activity of a polypeptide of claim 11 comprising:
  - (a) incubating a first sample of the polypeptide and its substrate;

-73-

- (b) measuring an uninhibited reactivity of the polypeptide from step (a);
- (c) incubating a first sample of the polypeptide and its substrate in the presence of a second sample comprising an inhibitor compound;
- (d) measuring an inhibited reactivity of the polypeptide from step (c); and,
- (e) comparing the inhibited reactivity to the uninhibited reactivity of the polypeptide.

20. A method for identifying compounds which bind to and inhibit an activity of a polypeptide of claim 11 comprising:

- (a) contacting a cell expressing on the surface thereof a binding site for the polypeptide, said binding being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said binding site, with a compound to be screened under conditions to permit binding to the binding site; and
- (b) determining whether the compound binds to and activates or inhibits the binding by detecting the presence or absence of a signal generated from the interaction of the compound with the binding site.

21. A method for identifying inhibitor-resistant AdT mutants comprising:

- (a) incubating a first sample of wild-type AdT and its substrate in the presence of a second sample comprising an AdT inhibitor;
- (b) measuring an unmutated reactivity of the AdT from step (a);

-74-

- (c) incubating a first sample of a mutated AdT and its substrate in the presence of a second sample comprising an AdT inhibitor;
- (d) measuring a mutated reactivity of the mutated AdT from step (c); and,
- (e) comparing the mutated reactivity to the unmutated reactivity of the wild-type AdT.

22. A method for inducing an immunological response in a mammal which comprises inoculating the mammal with amidotransferase, or a fragment or variant thereof, adequate to produce antibody to protect said animal from disease.

23. A method of inducing immunological response in a mammal which comprises, through gene therapy, delivering a gene encoding an amidotransferase fragment or a variant thereof, for expressing amidotransferase, or a fragment or a variant thereof *in vivo* in order to induce an immunological response to produce antibody to protect said animal from disease.

24. An immunological composition comprising a DNA which codes for and expresses an amidotransferase polynucleotide or protein coded therefrom which, when introduced into a mammal, induces an immunological response in the mammal to a given amidotransferase polynucleotide or protein coded therefrom.

-75-

25. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
  - (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising SEQ ID NO:4;
  - (b) a polynucleotide which is complementary to the polynucleotide of (a); and,
  - (c) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a) or (b).
26. A vector comprising the polynucleotide of claim 25.
27. A host cell comprising the vector of claim 26.
28. A process for producing a polypeptide comprising expressing from the host cell of claim 27 a polypeptide encoded by said polynucleotide.
29. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid set forth in SEQ ID NO:4.
30. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 29.

-76-

31. An antagonist which inhibits the activity of the polypeptide of claim 29.
32. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
  - (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising SEQ ID NO:6;
  - (b) a polynucleotide which is complementary to the polynucleotide of (a); and,
  - (c) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a) or (b).
33. A vector comprising the polynucleotide of claim 32.
34. A host cell comprising the vector of claim 33.
35. A process for producing a polypeptide comprising expressing from the host cell of claim 34 a polypeptide encoded by said polynucleotide..
36. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid set forth in SEQ ID NO:6.

-77-

37. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 36.
38. An antagonist which inhibits the activity of the polypeptide of claim 36.
39. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
  - (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising SEQ ID NO:8;
  - (b) a polynucleotide which is complementary to the polynucleotide of (a); and,
  - (c) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a) or (b).
40. A vector comprising the polynucleotide of claim 39.
41. A host cell comprising the vector of claim 40.
42. A process for producing a polypeptide comprising expressing from the host cell of claim 41 a polypeptide encoded by said polynucleotide.

-78-

43. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid set forth in SEQ ID NO:8.
44. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 43.
45. An antagonist which inhibits the activity of the polypeptide of claim 43.
46. An isolated heterotrimeric protein comprising subunits A, B, and C, wherein:  
said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;  
said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;  
said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic

-79-

acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

47. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a protein of claim 46.

48. An isolated nucleic acid molecule that encodes a protein of claim 46.

49. A recombinant host that has been altered to contain a nucleic acid molecule of claim 48.

50. A method for producing an AdT protein comprising the step of culturing the host of claim 49 under conditions in which said introduced nucleic acid molecule is expressed.

51. A method to identify an agent that blocks translation, said method comprising the steps of:

- (a) contacting an agent with an AdT protein, or a subunit thereof; and
- (b) determining whether said agent binds to said AdT protein or said subunit;

wherein said translation blocking agent is identified as being able to bind to said AdT protein, or said subunit.

-80-

52. The method of claim 51, wherein said AdT protein comprises a heterotrimeric protein consisting of an A, B and C subunit, wherein:

    said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

    said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

    said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

53. The method of claim 52, wherein a single subunit of said AdT protein is used, and:

    if subunit A is used, said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a

-81-

conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

if subunit B is used, said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

if subunit C is used, said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

54. The method of claim 51, wherein said agent is further tested for the ability to block the activity of said AdT protein.

55. The method of claim 54, wherein said AdT activity is tested in a cell free system.

-82-

56. The method of claim 54, wherein said AdT activity is tested in a cellular system.

57. A method to identify an agent that blocks translation, said method comprising the steps of:

- (a) contacting an agent with one or more of the subunits of an AdT protein;
- (b) incubating the three subunits of an AdT protein under conditions in which said subunits would associate to form an active AdT protein, wherein at least one of said subunits is from step (a);
- (c) determining whether said agent blocks the association of said three subunits;

wherein said translation blocking agent is identified as being able to block the association of the subunits of said AdT protein.

58. The method of claim 57, wherein said AdT protein comprises a heterotrimeric protein consisting of an A, B and C subunit, wherein:

    said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

-83-

    said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

    said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

59.    The method of claim 57, wherein said agent is further tested for the ability to block the activity of said AdT protein.

60.    The method of claim 59, wherein said AdT protein activity is tested in a cell free system.

61.    The method of claim 59, wherein said AdT protein activity is tested in a cellular system.

62.    A method to identify an agent that blocks translation, said method comprising the steps of:

-84-

- (a) contacting an agent with an AdT protein;
- (b) determining whether said agent blocks the activity of said AdT protein.

63. The method of claim 62, wherein said AdT protein comprises a heterotrimeric protein consisting of an A, B and C subunit, wherein:

    said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

    said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

    said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

-85-

64. The method of claim 63, wherein said AdT protein activity is tested in a cell free system.

65. The method of claim 63, wherein said AdT protein activity is tested in a cellular system.

66. A method to block translation of a protein within a cell, comprising the step of contacting said cell with an amount of an agent that binds to an AdT protein, or a subunit thereof, sufficient to block said translation.

67. The method of claim 66, wherein said agent binds to a subunit of said AdT and blocks the association of said subunits.

68. The method of claim 66, wherein said agent is used as an antibacterial agent.

69. The method of claim 66, wherein said agent is used as an antifungal agent.

70. The method of claim 66, wherein said agent is used as a herbicide.

71. An isolated polynucleotide that codes for a mutant AdT which confers resistance to an inhibitor of wild-type AdT.

-86-

72. A vector comprising the polynucleotide of claim 71.

73. A host cell comprising the vector of claim 72.

74. The host cell of claim 73 wherein the host cell comprises a plant cell.

75. A process for producing a polypeptide comprising expressing from the host cell of claim 73 a polypeptide encoded by said polynucleotide.

76. A process for producing a cell which expresses a polypeptide comprising transforming or transfecting the cell with the vector of claim 73 such that the cell expresses the polypeptide encoded by the polynucleotide contained in the vector.

77. A process for producing a plant which comprises a gene for resistance to an AdT inhibitor, said process comprising regenerating a plant from the plant cell of claim 74.

78. A process of plant husbandry comprising:

- (a) planting a plant which comprises a gene for resistance to an AdT inhibitor;
- (b) applying a herbicide which comprises an AdT inhibitor.

1/4



FIG. 1

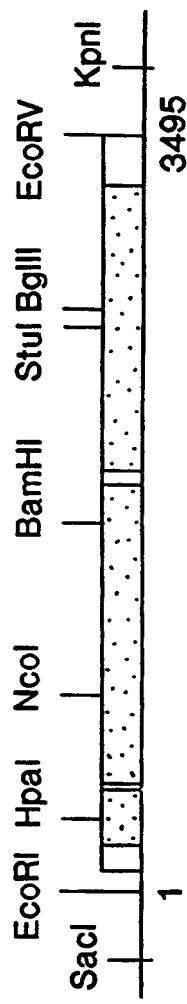
pABC (pBluescript KS-ABC, 6.4kb, Ap<sup>r</sup>)

FIG. 2

SUBSTITUTE SHEET (RULE 26)

2 / 4

1	<u>GAATT</u> CGATC	CTGTCTCAAG	GCGTTTTGTT	GCTTTAAAGG	GCTTGTTTT
	(ECORI)				
51	GATATGATCA	GTATTATATG	ACTTAACGGA	GAAATATGTG	GAGGTGGATC
	C				
	START→				
101	ATATGTCACG	AATTTCATA	GAAGAAGTAA	AGCACGTTGC	GCACCTTGCA
151	AGACTTGCAG	TTACTGAAGA	AGAAGCAAAA	ATGTTCACTG	AACAGCTCGA
201	CAGTATCATT	TCATTTGCCG	AGGAGCTTAA	TGAGGTTAAC	ACAGACAATG
251	TGGAGCCTAC	AACTCACGTG	CTGAAAATGA	AAAATGTCAT	GAGAGAAGAT
301	GAAGCGGGTA	AAGGTCTTCC	GGTTGAGGAT	GTCATGAAAA	ATGCGCCTGA
351	CCATAAAAGAC	GGCTATATTG	GTGTGCCATC	AATTCTGGAC	TAAAGGAGGG
	A				
	START→				
401	ACACAAGAAT	GTCATTATTT	GATCATAAAA	TCACAGAATT	AAAACAGCTC
451	ATACATAAAA	AAGAGATTAA	GATTCTGAT	CTGGTTGATG	AATCTTATAA
501	ACGCATCCAA	GCGGTTGATG	ATAAGGTACA	AGCCTTTTG	GCATTAGATG
551	AAGAAAGACG	CGCGGCATAC	GCAGAAGGAGC	TTGATGAGGC	GGTTGACGGC
601	CGTTCTGAGC	ACGGTCTTCT	TTTCGGTATG	CCGATCGGCG	TAAAAGATAA
651	TATCGTAACA	AAAGGGCTGC	GCACAACATG	CTCCAGCAAA	ATTCTCGAAA
701	ACTTTGATCC	GATTACGAT	GCTACTGTCG	TTCAGCGCCT	TCAAGACGCT
751	GAAGCGGTCA	CAATCGGAAA	ACTGAACATG	GACGAATTG	CCATGGGTC
801	ATCTACAGAA	AACTCAGCTT	ACAAGCTGAC	GAAAAAACCT	TGGAACCTGG
851	ATACAGTTCC	CGGCGGTTCA	AGCGGCGGAT	CTGCAGCTGC	GGTTGCTGCG
901	GGAGAAAGTTC	CGTTTTCTCT	TGGATCTGAC	ACAGGCGGCT	CCATCCGTCA
951	GCCGGCATCT	TTCTGCGGCG	TTGTCGGATT	AAAACCTACA	TACGGACGTG
1001	TATCTCGTTA	CGGCCTGGTC	GCATTTGCGT	CTTCATTGGA	CCAAATCGGA
1051	CCGATTACAC	GTACGGTTGA	GGATAACGCG	TTTTTACTTC	AAGCGATTTC
1101	CGGCGTAGAC	AAAATGGACT	CTACGAGTGC	AAATGTGGAC	GTGCCTGATT
1151	TTCTTTCTTC	ATTAACCTGGC	GACATCAAAG	GAATGAAAAT	CGCCGTTCCG
1201	AAAGAATAACC	TTGGTGAAGG	TGTCGGAAA	GAAGCGAGAG	AATCTGTCTT
1251	GGCAGCGCTG	AAAGTCCTTG	AAGGTCTCGG	CGCTACATGG	GAAGAAGTGT
1301	CTCTTCCGCA	CAGTAAATAC	GCGCTTGCAG	CATATTACCT	GCTGTCATCT

## FIG. 3A

SUBSTITUTE SHEET (RULE 26)

3/4

1351	TCTGAAGCGT	CAGCGAACCT	TGCACGCTTT	GACGGCATCC	GCTACGGCTA
1401	CCGCACAGAC	AACGCGGATA	ACCTGATCGA	CCTTTACAAG	CAAACGCGCG
1451	CTGAAGGTTT	CGGAAATGAA	GTCAAACGCC	GCATCATGCT	CGGAACGTTT
1501	GCTTTAAGCT	CAGGCTACTA	CGATGCGTAC	TACAAAAAAG	CGCAAAAAGT
1551	GCGTACGTTG	ATTAAGAAGG	ATTCGAGGA	CGTATTTGAA	AAATATGATG
1601	TTATTGTTGG	ACCGACTACA	CCGACACCTG	CGTTTAAAAT	CGGTGAAAAC
1651	ACGAAGGATC	CGCTCACAAT	GTACGCAAAC	GATATCTTAA	CGATTCCGGT
1701	CAACCTTGC	GCGTACCGGG	AATCAGGTGC	CATGCGGTTA	GCAGACGGAC
1751	TTCCGCTCGG	CCTGCAAATC	ATCGGAAAAC	ACTTTGATGA	AGCACTGTAT
1801	ACCGCGTTGC	TCATGCATTT	GAACAAGCAA	CAGACCATCA	TAAAGCAAAA
			B START→		
1851	CCTGAACGT	AAGGGGTGAA	AAGAATTGAA	CTTGAAACG	GTAATCGGAC
1901	TTGAAGTCCA	CGTTGAGTTA	AAAACAAAAT	AAAAAATTTT	CTCAAGCTCT
1951	CCAACGCCAT	TCGGCGCGGA	GGCGAATACG	CAGACAAGCG	TTATTGACCT
2001	CGGATATCCG	GGCGTCCTGC	CTGTTCTGAA	CAAAGAAGCC	GTTGAATTCG
2051	CAATGAAAGC	CGCTATGGCG	CTCAACTGTG	AGATCGCAAC	GGATACGAAG
2101	TTTGACCGCA	AAAACTATTT	CTATCCTGAC	AACCCGAAAG	CGTATCAGAT
2151	TTCTCAATT	GATAAGCCAA	TCGGCGAAA	CGGCTGGATC	GAAATTGAAG
2201	TCGGCGGCAA	AACAAAACGC	ATCGGCATCA	CGCGCCTTCA	TCTTGAAGAG
2251	GATGCCGAA	AACTGACGCA	TACGGGCGAC	GGCTATTCTC	TTGTTGACTT
2301	CAACCGTCAA	GGAACGCCG	TTGTTGAGTN	CGTATCAGAG	CCGGACATCC
2351	GCACGCCGGA	AGAACCGTAC	GCATATCTT	AAAAGCTGAA	ATCCATCATC
2401	CAATATACAG	GCGTTCTGA	CTGTAAAATG	GAAGAAGGCT	CACTTCGCTG
2451	TGACGCCAAT	ATCTCTCTTC	GTCCGATCGG	CCAAGAGGAA	TTCGGCACAA
2501	AAACAGAATT	GAAAAAACTTG	AACTCCTT	CGTTTGTCA	AAAAGGCCTT
2551	GAGCATGAAG	AAAAACGCCA	GGAGCAGGTT	CTTCTTCCG	GCTTCTTCAT
2601	CCAGCAAGAA	ACTCGCCGTT	ATGATGAAGC	AACGAAGAAA	ACCATTCTTA
2651	TGCGTGTCAA	AGAGGGATCT	GACGACTACC	GTIACCTCCC	AGAGCCAGAT

**FIG. 3B****SUBSTITUTE SHEET (RULE 26)**

4/4

2701	CTAGTCGAGC	TCTACATTGA	TGATGAATGG	AAGGAACGCG	TAAAAGCAAG
2751	CATTCCCTGAG	CTTCCGGATG	AGCGCCGCAA	GCGTTATATC	GAAGAGCTTG
2801	GCTTCGCTGC	ATATGACGCA	ATGGTTCTGA	CGCTGACAAA	AGAAATGGCT
2851	GATTTCCTCG	AAGAAACCGT	TCAAAAAGGC	GCTGAAGCTA	AACAAGCGTC
2901	TAACCTGGCTG	ATGGGTGAAG	TGTCAGCTTA	CCTAAACGCA	GAACAAAAAG
2951	AGCTTGCCGA	TGTTGCCCTG	ACACCTGAAG	GCCTTGCCGG	CATGATCAAA
3001	TTGATTGAAA	AAGGAACCAT	TTCTTCTAAG	ATCGCGAAGA	AAGTGTAA
3051	AGAATTGATT	GAAAAAGGCG	GCGACGCTGA	GAAGATTGTG	AAAGAGAAAG
3101	GCCTTGTTC	GATTTCCTGAC	GAAGGCGTGC	TTCTGAAGCT	TGTCACTGAG
3151	GCGCTTGACA	ACAATCCTCA	ATCAATCGAA	GACTTTAAAAA	ACGGAAAAGA
3201	CCGGCGGATC	GGCTTCCTAG	TCGGACAGAT	TATGAAAGCG	TCCAAAGGAC
3251	AAGCCAACCC	GCCGATGGTC	AACAAAATTC	TGCTTGAAGA	AATTAAAAAA
3301	CGCTAATAAA	AAAGCAGCCC	TTAGAGGCTG	CTTTTTTTAT	GGTCAAATTG
3351	AGATAAAAGAC	AAGATGAGGG	CCCGAAGCCT	TTCAACTTCT	TTGTCGTTGG
3401	TTCCGGCCAA	ATTGGACAGC	ATGCCTTTAT	AATCGGCTTG	CGCGGTTTAT
3451	CCTGAGTC	TTCTTCCTCG	ATAAGATAAG	TGACACGGTG	ATATC 3495 (EcoRV)

## FIG. 3C

# INTERNATIONAL SEARCH REPORT

Internat:	Application No
PCT/US 98/01860	

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 6 C12N15/54 C12N9/10 C07K16/40 C12Q1/52

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>KIM, S.I., ET AL.: "Bacillus subtilis PET112-like protein gene, complete cds" EMBL ACCESSION NO. U49790, 8 June 1996, XP002066349 see the whole document</p> <p>---</p> <p>EP 0 191 221 A (UNIV CALIFORNIA) 20 August 1986 see page 16, line 22 - page 19, line 18</p> <p>---</p> <p>-/-</p>	1-4,6,7, 11, 25-27, 32-34,36
X		1

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

11 June 1998

02-07- 1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Maddox, A

## INTERNATIONAL SEARCH REPORT

Application No  
PCT/US 98/01860

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CURNOW, ALAN W. ET AL: "Glu-tRNAGln amidotransferase: a novel heterotrimeric enzyme required for correct decoding of glutamine codons during translation" PROC. NATL. ACAD. SCI. U. S. A. (1997), 94(22), 11819-11826 , XP002066350 see the whole document	1-3, 5-12, 25-29, 32-37, 39-44, 46-50
P,X	-& CURNOW, A.W., ET AL.: "Bacillus subtilis Glu-tRNAGln amidotransferase subunits C (gatC). A (gatA) and B (gatB) genes complete cds." EMBL ACCESSION NO.AF008553, 18 November 1997, XP002066351 see the whole document	1-9,11, 25-29, 32-36, 39-43
P,X	---	
P,X	CURNOW, ALAN W. ET AL: "tRNA-dependent amino acid transformations" NUCLEIC ACIDS SYMP. SER. (1997), 36(SYMPORIUM ON RNA BIOLOGY, II. RNA: TOOL AND TARGET, 1997), 2-4 , XP002066352 see the whole document	11,29, 36,43,46
P,X	---	
P,X	EP 0 786 519 A (HUMAN GENOME SCIENCES INC) 30 July 1997 see sequence IDs154, 4168, 2974, 3085, 3954, 2987.	1-3,6,7, 32-34
A	---	
A	WILCOX, M., ET AL.: "Transfer RNA as a cofactor coupling amino acid synthesis with that of protein" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 61, 1968, WASHINGTON US, pages 229-236, XP002066353 see the whole document	1-78
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A	STRAUCH, M.A., ET AL.: "Characterization of the glutamyl-tRNAGln-to-glutaminyl-tRNAGln amidotransferase reaction of <i>Bacillus subtilis</i> " JOURNAL OF BACTERIOLOGY, vol. 170, 1988, pages 916-922, XP002066354 see the whole document	1-78
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A	JAHN D ET AL: "PURIFICATION AND FUNCTIONAL CHARACTERIZATION OF THE GLUTAMINE GLUTAMINYL TRANSFER RNA AMIDOTRANSFERASE FROM CHLAMYDOMONAS-REINHARDTII." J BIOL CHEM 265 (14). 1990. 8059-8064. , XP002066355 see the whole document	1-78
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## INTERNATIONAL SEARCH REPORT

International Application No	
PCT/US 98/01860	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CURNOW, ALAN W. ET AL: "tRNA-dependent asparagine formation" NATURE (LONDON) (1996), 382(6592), 589-590 , XP002066356 see the whole document ---	1-78
A	ZALKIN, HOWARD: "Glu-tRNAGln amidotransferase" METHODS ENZYMOL. (1985), 113(GLUTAMATE, GLUTAMINE, GLUTATHIONE, RELAT. COMPD.), 303-5 , XP002066357 see the whole document ---	1-78
A	TABATA, S.: "Synechocystis sp. PCC6803 complete genome, 9/27, 1056467-1188885" EMBL ACCESSION NO. D90907, 31 October 1996, XP002067229 see sequences 79301-80700 ---	25
A	WENGENDER, P.A., ET AL.: "Identification of a putP proline permease gene homolog from <i>Staphylococcus aureus</i> by expression cloning of the high-affinity proline transport system in <i>Escherichia coli</i> " APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 61, no. 1, January 1995, pages 252-259, XP002067230 see fig 3 sequence positions 200-500 ---	39
A	SCHOEN, ASTRID ET AL: "Protein biosynthesis in organelles requires misaminoacetylation of tRNA" NATURE (LONDON) (1988), 331(6152), 187-90 , XP002067757 see the whole document -----	74,77,78

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International application No.  
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### Box I Observation where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
Although claims 14,15,16,22,23,66-70 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US 98/01860

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP 0191221 A	20-08-1986	US	4816405 A	28-03-1989
EP 0786519 A	30-07-1997	CA	2194411 A	06-07-1997
		JP	9322781 A	16-12-1997

